

Improved outcome of treatment-resistant high-risk Langerhans cell histiocytosis after allogeneic stem cell transplantation with reduced-intensity conditioning

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Summary:

Children with multisystem Langerhans cell histiocytosis (LCH) and risk organ involvement who fail to respond to conventional chemotherapy have an extremely poor prognosis. Myeloablative stem cell transplantation (SCT) as a possible salvage approach for these patients has been associated with a high risk of transplant-related mortality. Therefore, allogeneic stem cell transplantation following a reduced-intensity conditioning regimen (RIC-SCT) has recently been performed as an alternative salvage approach. We report on the experience with allogeneic RIC-SCT in nine pediatric high-risk LCH patients. Conditioning regimen included fludarabine in all patients, melphalan in eight patients, total lymphoid irradiation in six patients, total body irradiation in two, antithymocyte globulin in five, and Campath in four patients. RIC-SCT was well tolerated with regard to common procedure-related complications. Two patients died 50 and 69 days after RIC-SCT, respectively. Seven out of the nine patients survived and showed no signs of disease activity (including one with nonengraftment and full autologous hematopoietic recovery) after median follow-up of 390 days post-SCT. Based on this observation, we conclude that RIC-SCT is a feasible procedure with low transplant-related morbidity and mortality and a promising new salvage approach for high-risk LCH patients with resistant risk organ involvement.

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Langerhans cell histiocytosis (LCH) is a rare disorder, characterized by an accumulation and proliferation of histiocytic cells displaying the phenotype of the patho-

logical Langerhans cells.¹ The etiology is still unknown, but it has recently become evident that a deranged immunological crosstalk involving the LCH cells and T cells, leading to abundant cytokine production, plays a crucial role in the pathogenesis of this disease.^{2,3} The clinical manifestation pattern of LCH ranges from single-system disease, mostly affecting the bones or the skin and often requiring minimal therapeutic interventions only, to severe and sometimes life-threatening multisystem disease (MS-LCH).

In particular, MS-LCH patients with involvement of 'risk organs' (ie hematopoietic system, liver, spleen, lungs) and failure of conventional therapy have a very poor outcome with survival rates of about 20%.^{4–6} These 'high-risk' patients comprise about 20% of the whole MS-LCH population and have been found in several studies not to be curable with conventional chemotherapeutic regimens.⁵ During the last decade, no therapeutic progress towards an improved outcome could be achieved in this particular patient cohort,⁷ as neither intensification of the chemotherapy nor different salvage approaches, including monotherapy with cyclosporin A (CSA)⁸ and 2-CdA, resulted in improved survival rates.

Several cases of complete and sustained remission following allogeneic stem cell transplantation (SCT) have been reported.^{9–21} However, myeloablative high-dose regimens are associated with a high treatment-related morbidity and mortality, especially in severely ill LCH patients. For children with other nonmalignant diseases who are not eligible for conventional myeloablative conditioning regimens, the use of reduced-intensity conditioning regimens is feasible and associated with stable engraftment and cure from the underlying disease in many cases.^{22,23} In this paper, we report on the experience with allogeneic SCT following reduced-intensity conditioning (RIC-SCT) in nine pediatric patients with high-risk MS-LCH in Austria, Germany and France.

Patients and methods

Patients

Nine high-risk LCH patients, who have undergone RIC-SCT in Austria, Germany and France since May 2000, were

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reported to the Study Reference Center of the International LCH Study at St Anna Children's Hospital. Three patients (two from Italy and one from Greece) underwent SCT at St Anna Children's Hospital in Vienna, Austria. Relevant information on the other six patients, who underwent SCT in different centers in Germany and France, was provided by the respective institution on appropriate questionnaires and medical reports. Data from one patient were previously published.²⁴

Diagnostic criteria and definition of disease state

In all patients, histopathological diagnosis of LCH was established according to standard criteria.²⁵ The extent of disease was assessed according to a baseline evaluation, and any abnormalities led to specialized extended evaluation.²⁶ Hematopoietic involvement was diagnosed upon the presence of dysfunction criteria, that is, anemia (<10 g/dl hemoglobin, or <9 g/dl hemoglobin in infants, not due to iron deficiency or to infection) and/or thrombocytopenia (platelets $<100\,000/\mu\text{l}$) and/or leukopenia (white blood count $<4000/\mu\text{l}$).²⁶ Involvement and dysfunction of liver, spleen or lungs was also defined as reported previously.²⁶

For the definition of disease state and response to treatment, we employed the criteria of the international LCH-1 study.⁵ Complete resolution was defined as 'non-active disease' (NAD), a regression of disease as 'active disease better' (AD better). A progression of disease was classified as 'active disease worse' (AD worse), whereas no change in disease activity was judged as 'active disease stable' (AD stable). Reactivation was defined as reappearance of disease activity after complete resolution of all signs and symptoms.

Clinical course prior to SCT

Patient characteristics and the clinical course prior to SCT are shown in Table 1. There were five males and four females, with a median age at diagnosis of LCH of 9.9 months (range 1.4–22 months). The disease extent and organ involvement was evaluated twice, first at initial diagnosis and second within 4 weeks prior to SCT. At diagnosis, hematopoietic dysfunction was present in all children, with a median hemoglobin of 8 g/dl and a median platelet count of $67\,000/\mu\text{l}$. All patients received standard first-line LCH therapy according to the study protocol LCH-2 or LCH-3 of the Histiocyte Society²⁷ and experienced severe disease progression unresponsive to chemotherapy, either in the course of initial disease ($n=7$) or upon first reactivation ($n=2$). The main features of disease progression in the nine patients were progressive hepatosplenomegaly, persistent fever and increasing signs of organ dysfunction of the hematopoietic system ($n=9$), the liver ($n=5$) or the lungs ($n=3$).

In one patient (pt. 8), RIC-SCT had to be performed in an intensive-care setting due to severe LCH-related pulmonary dysfunction and requirement for assisted ventilation. In addition, this patient presented with a cirrhotic liver transformation at diagnosis of LCH, and was detected to be a heterozygote carrier of the alpha-1-antitrypsin deficiency allele PiZ.

Conditioning regimen and SCT

The transplant characteristics of the nine patients are summarized in Table 2. The median time interval from initial diagnosis to SCT was 350 days (range 124–637 days), and median age at SCT was 20 months (range 14–30 months). Overall, 12 SCTs were performed in the nine patients. Three patients (pts. 1, 2 and 7) underwent a second SCT due to primary graft rejection. One of them (pt. 1) was initially transplanted according to the Slavin protocol.²⁸ As this protocol is different to the RIC-SCT procedure presented herein, the data of this patient's 1st transplant are not included in this analysis.

The reduced-intensity conditioning regimen applied is summarized in Table 2 and basically consisted of six consecutive doses of fludarabine (cumulative dosage 90–180 mg/m²), one dose of melphalan (140 mg/m²), three consecutive doses of antithymocyte globulin (cumulative dosage 7.5–90 mg/kg) and a total lymphoid irradiation (TLI) (cumulative dosage 2–5 Gy). In four patients (pt. 2 (2nd SCT), pts. 6, 7 (1st SCT), 9), MabCampath (anti-CD52 antibody) was included in the conditioning regimen (cumulative dosage 0.6–1 mg/kg), in two of them (pts. 6 and 9) in replacement for TLI. Total body irradiation (TBI) (cumulative dosage 2 Gy) was given to two patients (pt. 2 (2nd SCT), pt. 8).

Four patients, undergoing a total of five RIC-SCT procedures (pt. 1 (2nd SCT), pt. 2 (1st and 2nd SCT), pt. 7 (1st SCT), pt. 8), received T-cell-depleted peripheral stem cells from haploidentical parental donors ($n=3$ pts.) and a 1-antigen (1-AG) mismatch unrelated donor ($n=1$ pt.) with a median of $28.1 \times 10^6/\text{kg}$ CD34+ cells (range $18\text{--}47.7 \times 10^6/\text{kg}$). Four patients (pts. 4, 5, 6 and 9) received unmanipulated bone marrow from matched sibling donors ($n=3$) and a matched unrelated donor ($n=1$) with a median of $13 \times 10^6/\text{kg}$ CD34+ cells (range $8.05\text{--}16 \times 10^6/\text{kg}$). One patient (pt. 7 (2nd SCT)) received red cell-depleted bone marrow from a 1-AG mismatch unrelated donor ($3.8 \times 10^6/\text{kg}$ CD34+ cells) and one patient undergoing 1-AG mismatch cord blood transplantation received $6.27 \times 10^7/\text{kg}$ nucleated cells.

Graft-versus-host disease (GvHD) and rejection prophylaxis

Prophylaxis for GvHD and graft rejection consisted of CSA, mycophenolate mofetil (MMF) and prednisone in four patients and CSA with either prednisone, MMF or methotrexate (MTX) in five patients, with doses of 3–6 mg/kg for CSA, 30 mg/kg for MMF and 1–3 mg/kg for prednisone.

Engraftment

Engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) of $500/\mu\text{l}$ or greater and the evidence of donor cell origin in at least one cell line by chimerism analysis. Nonengraftment was defined as the constant absence of donor cells followed by a complete autologous hematopoietic reconstitution. Graft rejection was considered when donor cells were detected in

Table 1 Patient characteristics and clinical course prior to nonmyeloablative SCT

| | <i>Sex</i> | <i>Age at diagnosis</i> | <i>Clinical signs and symptoms at diagnosis</i> | <i>First-line therapy</i> | <i>Treatment response at week 12</i> | <i>Course of LCH prior to RIC-SCT</i> | <i>Second-line therapy (prior to RIC-SCT)</i> | <i>Clinical signs and symptoms within 4 weeks prior to SCT</i> | <i>Interval from diagnosis to SCT (days)</i> |
|-------|------------|-------------------------|--|---------------------------|--------------------------------------|---------------------------------------|---|--|--|
| pt. 1 | M | 4 mo | Fever, hepatosplenomegaly, hematopoietic dysf., skin | Pred, Vbl, VP-16 | AD stable | Progression of initial disease | 2-CdA, SCT (Slavin-protocol) | Fever, progressive hepatosplenomegaly, hematopoietic dysf., skin | 516 |
| pt. 2 | M | 22 mo | Fever, hepatosplenomegaly, hematopoietic dysf., skin, bone | Pred., Vbl | AD worse | Progression of initial disease | Pred., Vbl, 6-MP, MTX | Fever, progressive hepatosplenomegaly, hematopoietic dysf., liver dysf., edema, skin, bone | 124 |
| pt. 3 | M | 1.7 mo | Fever, hepatosplenomegaly, hematopoietic dysf., lung dysf., skin | Pred, Vbl, MTX, 6-MP | AD better | Progression of initial disease | Pred, Vbl, MTX, VP-16 | Fever, progressive hepatosplenomegaly, hematopoietic dysf., liver dysf., edema, lung dysf., skin | 406 |
| pt. 4 | M | 12.6 mo | Fever, hepatosplenomegaly, hematopoietic dysf., skin | Pred, Vbl, VP-16, 6-MP | NAD | Reactivation | Pred, Vbl, MTX, 6-MP, 2-CdA | Fever, progressive hepatosplenomegaly, hematopoietic dysf., skin, bone | 531 |
| pt. 5 | M | 1.4 mo | Hepatosplenomegaly, hematopoietic dysf., skin | Pred, Vbl, 6-MP, MTX | NAD | Reactivation | Pred, Vbl, MTX, etanercept | Fever, progressive hepatosplenomegaly, hematopoietic dysf., lung dysf., skin, bone | 637 |
| pt. 6 | F | 12.4 mo | Fever, hepatosplenomegaly, hematopoietic dysf., liver dysf., ascites, GI tract, skin, bone | Pred, Vbl, VP-16 | AD worse | Progression of initial disease | Pred, Vbl, 6-MP | Fever, progressive hepatosplenomegaly, hematopoietic dysf., liver dysf., ascites, skin, bone | 124 |
| pt. 7 | F | 5.8 mo | Hematopoietic dysf., skin, bone | Pred, Vbl, 6-MP | AD worse | Progression of initial disease | Pred, Vbl, MTX, 2-CdA, Campath | Fever, progressive hepatosplenomegaly, hematopoietic dysf., liver dysf., skin, bone | 255 |
| pt. 8 | F | 9.9 mo | Fever, hepatosplenomegaly, liver dysf. (+cirrhosis!), ascites, GI tract, hematopoietic dysf., skin | Pred, Vbl, MTX, 6-MP | AD better | Progression of initial disease | 2-CdA, Ara-C, etanercept | Fever, progressive hepatosplenomegaly, hematopoietic dysf., liver dysf. (cirrhosis), lung dysf. requiring CPAP ventilation, skin | 350 |
| pt. 9 | F | 11 mo | Fever, hepatosplenomegaly, hematopoietic dysf., lung dysf., GI tract, bone | Pred, Vbl, MTX | AD worse | Progression of initial disease | 2-CdA, Ara-C | Fever, hepatosplenomegaly, hematopoietic dysf., lung dysf., GI tract, bone | 215 |

dysf. = dysfunction; GI tract = gastrointestinal tract; AD = active disease; NAD = non active disease; Pred = prednisone; Vbl = vinblastine; VP-16 = etoposide; 2-CdA = 2-chlorodeoxyadenosine; 6-MP = 6-mercaptopurine; MTX = methotrexate.

Table 2 Transplant characteristics of the nine patients

| | Age at SCT | Donor | Stem cells: source, manipulation, CD34 cells/kg | Conditioning regimen | GvHD and rejection prophylaxis | Engraftment | ANC >500/ μ l (in days from SCT) | Date of last transfusion | Donor lymphocyte infusion | Chimerism at last follow-up | Relevant transplant-related toxicity | Relevant infections | GvHD |
|--------------------|------------|-------------------------------|---|---|--------------------------------|-----------------------------------|--------------------------------------|--|--|--|--|--|---|
| pt. 1 ^a | 21 mo | Haploidentical mother | PBSC T-cell depleted 27×10^6 | BU (8 mg/kg) FLU (180 mg/m ²) ATG (15 mg/kg) | CSA MMF Prednisone | Yes (early rejection and 2nd SCT) | +40 | — | — | Full autologous recovery | Mucositis | Bacterial sepsis | No |
| | 24 mo | Haploidentical father | PBSC T-cell depleted 28.1×10^6 | TLI (5 Gy) FLU (180 mg/m ²) MEL (140 mg/m ²) ATG (20 mg/kg) | CSA MMF Prednisone | Yes | +17 | Death | Day +46 1×10^5 CD3/kg | Mixed chimerism | Mucositis (grade 3) | CMV-infection; bacterial sepsis | No |
| pt. 2 ^a | 26 mo | Haploidentical father | PBSC T-cell depleted 22.6×10^6 | TLI (2 Gy) FLU (180 mg/m ²) MEL (140 mg/m ²) ATG (7.5 mg/kg) | CSA MMF Prednisone | Yes (early rejection and 2nd SCT) | +14 | — | — | Full autologous recovery | Mucositis (grade 3) | Bacterial sepsis | No |
| | 27 mo | Haploidentical father | PBSC T-cell depleted 47.7×10^6 | TBI (2 Gy) FLU (90 mg/m ²) Camp (1 mg/kg) | CSA MMF Prednisone | Non-engraftment | +18 | Day +43 (red cells and platelets) | — | Full autologous recovery | Mucositis | Adenovirus-infection | No |
| pt. 3 | 15 mo | 1-AG mismatch unrelated donor | Cord blood 6.27×10^7 nucleated cells | TLI (2 Gy) FLU (180 mg/m ²) MEL (140 mg/m ²) ATG (7.5 mg/kg) | CSA MMF Prednisone | Yes | +49 | Day +201 (red cells) Day +447 (platelets) | — | Complete donor chimerism | Mucositis (grade 3); hypertension requiring drug therapy | CMV-disease with pneumonia; bacterial sepsis | aGvHD (grade 2-3) cGvHD (transient until day +145) |
| pt. 4 | 30 mo | HLA-identical brother | Bone marrow 15×10^6 | TLI (2 Gy) FLU (180 mg/m ²) MEL (140 mg/m ²) | CSA MMF | Yes | +13 | Day +57 (red cells) Day +69 (platelets) | — | Complete donor chimerism | Mucositis (grade 2) | CMV-reactivation | No |
| pt. 5 | 22 mo | HLA-identical unrelated donor | Bone marrow 8.05×10^6 | TLI (2 Gy) FLU (175 mg/m ²) MEL (140 mg/m ²) ATG (40 mg/kg) | CSA MMF | Yes | +13 | Day +32 (red cells) Day +20 (platelets) | Day +279 1×10^5 CD3/kg Day +307 5×10^5 CD3/kg | Mixed chimerism (decreasing donor signals) | Mucositis (grade 2) | CMV-reactivation HHV-6 infection; Salmonella enteritis | No |
| pt. 6 | 16 mo | HLA-identical sister | Bone marrow 16×10^6 | FLU (180 mg/m ²) MEL (140 mg/m ²) Camp (1 mg/kg) | CSA MMF Prednisone | Yes | +21 | Day +30 (red cells and platelets) | Day +66 1×10^7 CD3/kg | Mixed chimerism | Mucositis (grade 1) | No | No |

Table 2 Continued

| | <i>Age at SCT</i> | <i>Donor</i> | <i>Stem cells: source, manipulation, CD34 cells/kg</i> | <i>Conditioning regimen</i> | <i>GvHD and rejection prophylaxis</i> | <i>Engraftment</i> | <i>ANC > 500/μl (in days from SCT)</i> | <i>Date of last transfusion</i> | <i>Donor lymphocyte infusion</i> | <i>Chimerism at last follow-up</i> | <i>Relevant transplant-related toxicity</i> | <i>Relevant infections</i> | <i>GvHD</i> |
|--------------------|-------------------|--------------------------------------|--|--|---------------------------------------|----------------------------------|--|--|----------------------------------|------------------------------------|---|---|-------------|
| pt. 7 ^a | 14 mo | Haploidentical mother | PBSC T-cell depleted 18×10^6 | TLI (2 Gy) FLU (150 mg/m ²) MEL (140 mg/m ²) Camp (0.6 mg/kg) | CSA Prednisone | Yes (late rejection and 2nd SCT) | +14 | Day +61 (red cells) Day +71 (platelets) | — | Full autologous recovery | Mucositis (grade 2) | Bacterial sepsis | No |
| | 22 mo | 1-AG mismatch unrelated donor (9/10) | Bone marrow Red cell depleted 3.8×10^6 | FLU (180 mg/m ²) MEL (140 mg/m ²) ATG (60 mg/kg) | CSA MMF MTX | Yes | +20 | Day +21 (red cells), Day +20 (plts) | — | Complete donor chimerism | No | No | No |
| pt. 8 | 21 mo | 1-AG mismatch unrelated donor | PBSC T-cell depleted 30×10^6 | TBI (2 Gy) FLU (180 mg/m ²) MEL (140 mg/m ²) ATG (90 mg/kg) | CSA MTX | Yes | +23 | Death | — | Complete donor chimerism | Mucositis (grade 2) | CMV-disease with pneumonia; bacterial sepsis | No |
| pt. 9 | 18 mo | HLA-identical sister | Bone marrow 11×10^6 | FLU (150 mg/m ²) MEL (140 mg/m ²) Camp (1 mg/kg) | CSA MTX | Yes | +12 | Day +8 (red cells) Day +22 (platelets) | — | Complete donor chimerism | Mucositis (grade 2) | Prolonged adenovirus infection, HHV-infection | No |

1-AG mismatch = 1-antigen mismatch; PBSC = peripheral blood stem cells; BU = busulfan; FLU = fludarabine; MEL = melphalan; ATG = antithymocyte globulin; TLI = total lymph node irradiation; TBI = total body irradiation; Camp = Campath; CSA = cyclosporin A; MMF = mycophenolate mofetil; aGvHD = acute graft-versus-host disease; ANC = absolute neutrophil count; cGvHD = chronic graft-versus-host disease.

^apts. 1, 2 and 7 underwent two SCTs.

at least one cell subset, followed by subsequent recipient chimerism in all cell subsets before day +28 (early rejection) or thereafter (late rejection).

Chimerism

Chimerism was determined by polymerase chain reaction based on the amplification of short-tandem-repeat markers (STR-PCR) or variable nucleotide tandem repeat markers in eight patients (pts. 1–7 and 9). In one case of sex mismatch transplant, chimerism was assessed by XY chromosome fluorescence *in situ* hybridization analysis (pt. 8). The chimerism pattern was analyzed on a semiquantitative basis by defining three chimerism patterns: (1) recipient chimerism – no donor cells detectable by means of STR-PCR, (2) mixed chimerism – recipient and donor allelic signals detectable by STR-PCR and (3) full donor chimerism – no recipient allelic signals detectable by STR-PCR.

Follow-up

Follow-up information was regularly obtained either by examination in the transplantation unit of the St Anna Children's Hospital (pts. 1, 2 and 6) or by appropriate questionnaires and medical reports sent from the participating institutions (pts. 3–5 and 7–9).

GvHD was graded according to the Glucksberg criteria.²⁹ Disease state was defined as described above. Hepatic and hematopoietic function was assessed on day +100. Performance status at last follow-up was documented using the Lansky play scale.³⁰ Survival was estimated by the Kaplan–Meier method.³¹

Results

The clinical course after RIC-SCT with respect to engraftment, chimerism and transplant-related morbidity is demonstrated in Table 2.

Engraftment and chimerism

Following (first) RIC-SCT, primary engraftment with more than 500/ μ l ANC and donor chimerism in at least one cell population was seen in all patients. The median time to engraftment was 14 days (range 12–49 days) with the majority engrafting between 12 and 23 days. Late engraftment at day +49 was seen, as expected, in the patient with 1-AG mismatch cord blood transplantation (pt. 3). Two patients subsequently rejected the graft following haplo-identical RIC-SCT (pts. 2 and 7). One of them (pt. 2) had neutrophil engraftment at day +14 with a short period of detectable donor allelic signals, but subsequently experienced an early rejection with complete autologous hematopoietic recovery. This patient underwent a second haplo-identical RIC-SCT and showed nonengraftment with full autologous reconstitution of hematopoiesis. The other patient (pt. 7) showed regular engraftment at day +14 with full donor chimerism, but subsequently developed decreasing donor allelic signals, followed by late secondary graft

rejection 130 days post transplant. This patient underwent a second RIC-SCT from a 1-AG mismatch unrelated donor and had full donor chimerism at the last follow-up (100 days post 2nd RIC-SCT).

Overall, after (last) RIC-SCT, five of the nine patients had full donor chimerism at day +28 (pts. 3, 4 and 7–9), which persisted until the last follow-up (692, 581, 100, 69 and 390 days post-SCT, respectively).

In two patients (pt. 1 and 6), mixed chimerism remained unchanged until the last follow-up. One patient (pt. 5) showed decreasing donor chimerism and received donor lymphocyte infusions, which had not shown a major effect at the time point of last follow-up.

Seven of the nine patients (pts. 2–7 and 9) reached transfusion independence from platelets and red cells after a median of 37 and 38 days post transplantation, respectively (range for platelets 20–447 days; range for red cells 8–201 days). Two patients died before achievement of transfusion independence (pts. 1 and 8).

Graft-versus-host disease

One of the seven patients (pt. 3) with mismatch cord blood transplantation developed grade II–III acute GvHD involving the gastrointestinal mucosa, the liver and the skin, followed by limited chronic GvHD, both responsive to immunosuppressive therapy.

Transplant-related morbidity and mortality

Toxic and infectious complications are summarized in Table 2. Mucositis ranging between WHO grade I and III was the most common toxic side effect noted and was present in almost all patients. None of the patients developed veno-occlusive disease and the hepatic toxicity did not exceed WHO grade 3 and resolved in all cases. One patient (pt. 3) developed arterial hypertension and required antihypertensive drug therapy.

Viral infections were documented in seven patients. In one of them (pt. 8), cytomegalovirus (CMV)-associated pneumonia together with LCH-related lung dysfunction led to fatal deterioration of pulmonary function. Bacterial sepsis with positive blood cultures was seen in five patients. One of them died (pt. 1) due to a Gram-negative sepsis (*Klebsiella*) on day +50 following the second haplo-identical SCT.

Clinical recovery from the underlying disease after RIC-SCT

Recovery from the underlying disease was evaluated after SCT by clinical parameters of remission of hematopoietic dysfunction, regression of hepatosplenomegaly and remission of fever. Hematopoietic dysfunction, defined as stated above, was associated with persistent requirement of red cell and platelet transfusions in all patients during a broad time period up to SCT.

Two patients who died early (pts. 1 and 8) did not experience a comprehensible clinical recovery after RIC-SCT. In the remaining seven patients (pts. 2–7 and 9) hematopoietic function slowly recovered after (last) RIC-

SCT and sustained transfusion independence was reached after median of 38 days (Table 2). Also, hepatosplenomegaly gradually regressed over a median of 88 days (range 42–581 days) and fever subsided over median of 33 days (range 9–231 days) (see Table 3 and two illustrative cases in Figure 1).

Outcome

After a median follow-up of 390 days post transplant (range 215–881 days), seven out of the nine patients are alive and without any signs of active LCH disease (pts. 2–6, 7 and 9). Patient 2 initially rejected the haploidentical graft and underwent a second haploidentical RIC-SCT, which was again followed by nonengraftment and full autologous reconstitution of hematopoiesis. Under additional therapy post transplant, consisting of etanercept and 6-MP/MTX (see Table 3), he became disease free within 3 months after the 2nd SCT and is off any therapy and in excellent clinical condition without any signs of disease activity 770 days post transplant.

At the time of last follow-up, the Lansky play scale ranged between 70 and 100% (median 100%) in the seven survivors, with four of them being off any therapy (Table 3). The corresponding probability of survival was $78 \pm 14\%$.

Two patients died 50 and 69 days after RIC-SCT, respectively (pts. 1 and 8). Patient 1 engrafted following the second haploidentical SCT (RIC-SCT), but subsequently died after 50 days due to Gram-negative sepsis. Patient 8 with severe organ dysfunction prior to RIC-SCT (details above) subsequently developed a fatal acute respiratory

distress syndrome, which was supposed to result from the combination of LCH-related lung disease and a CMV-associated pneumonia in the presence of a CMV IgG-negative donor. Both patients who died had not shown a reduction of hepatosplenomegaly or a persistent remission of fever at any time point after transplantation.

Discussion

Megatherapy followed by SCT as a therapeutic option for LCH emerged in 1987, when an allogeneic BMT was successfully performed by Ringden *et al*⁹ in a 20-year-old male patient. Subsequently, a few cases of allogeneic SCT in LCH patients failing other therapeutic approaches have been published.^{10–21} In order to critically evaluate the published data on myeloablative allogeneic SCT, we exclusively reviewed publications concerning pediatric LCH patients with involvement of risk organs prior to SCT (Table 4). In all, 29 pediatric patients underwent myeloablative allogeneic SCT for LCH with risk organ involvement. Overall survival was 48% (14/29 patients), and the transplant-related mortality was exceedingly high with 45% (13/29 patients). Pre-existing disease-related hepatic, hematopoietic and pulmonary dysfunction together with a substantial toxic and infectious preload in the majority of the patients seemed to be the major cause for the high transplant-related morbidity and mortality. This high susceptibility to severe regimen-related toxic complications represented the major rationale for the

Table 3 Post transplant therapy, clinical recovery and outcome

| | Therapy post RIC-SCT (besides GvHD prophylaxis) | Regression of hep.splenomegaly/ remission of fever (in days post-SCT) | Outcome and therapy at last follow-up | Observation time (in days from 1st SCT) |
|-------|---|---|---|---|
| pt. 1 | No | — | Death day + 50 after RIC-SCT due to <i>Klebsiella</i> sepsis | — |
| pt. 2 | Etanercept until day +200 post-2nd SCT | 88/37 ^a | Alive, NAD, off therapy, Lansky play scale: 100% | 770 |
| pt. 3 | Oral 6-MP and MTX from day +200 until day +600 post-2nd SCT | 517/231 | Alive, NAD, off therapy, Lansky play scale: 100% | 881 |
| pt. 4 | None | 581/34 | Alive, NAD, off therapy, Lansky play scale: 100% | 710 |
| pt. 5 | None | 181/18 | Alive, NAD, off therapy, Lansky play scale: 100% | 310 |
| pt. 6 | Campath from day +1 to day +5 (1 mg/kg) | 42/33 | Alive, NAD therapy: GvHD prophylaxis, Lansky play scale: 70% | 215 |
| pt. 7 | None | 30/30 ^a | Alive, NAD, therapy: GvHD prophylaxis, Lansky play scale: 80% | 350 |
| pt. 8 | Etanercept | — | Death day + 69 due to pulmonary insufficiency (LCH disease and CMV infection) | — |
| pt. 9 | Immunoglobulins (persistent lymphopenia) | 87/9 | Alive, NAD, therapy: GvHD prophylaxis, immunoglobulins, Lansky play scale: 100% | 390 |

^aDays post-2nd SCT in patients 2 and 7.

NAD = non active disease; 6-MP = 6-mercaptopurine; MTX = methotrexate.

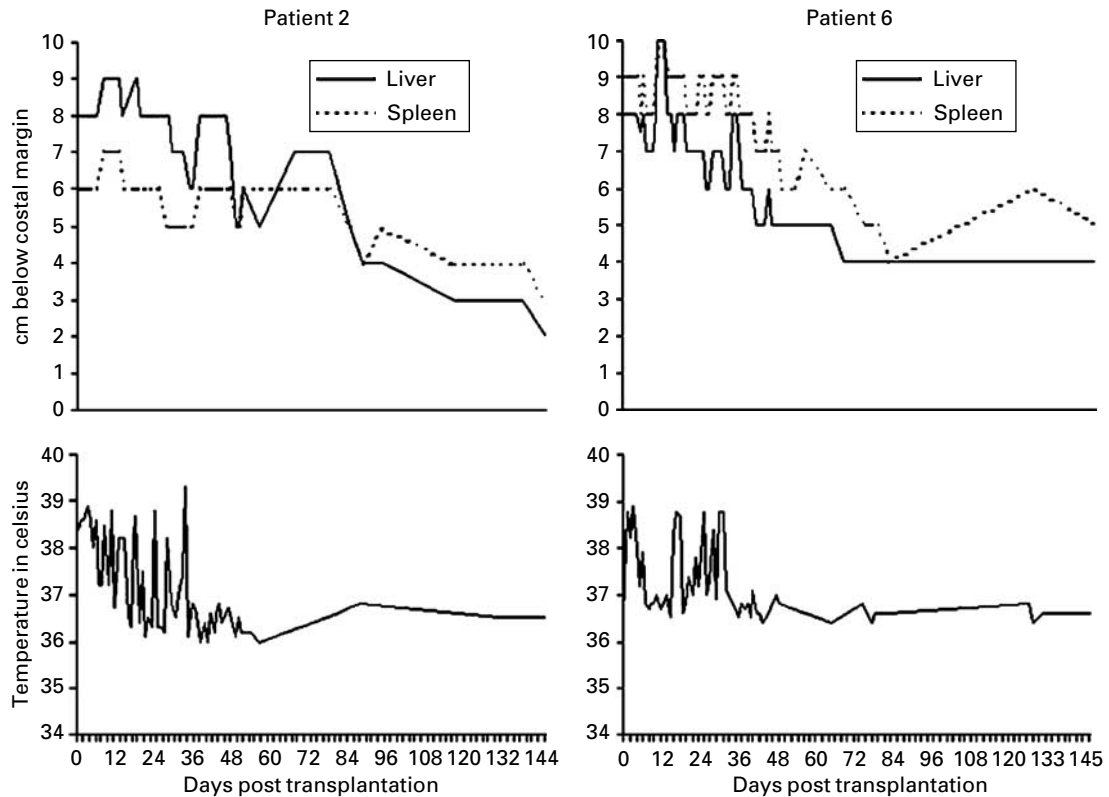


Figure 1 Post-transplant clinical recovery in two representative cases (pts. 2 and 6): the upper diagrams represent the course of hepatosplenomegaly and the lower diagrams the course of fever.

development of an intensity-reduced conditioning regimen for this patient population.

Further reasons to favor a nonmyeloablative transplantation procedure relate to the pathophysiologic mechanisms involved in LCH. Although the main etiologic issues of LCH still remain enigmatic and there is much debate whether the disease is basically a reactive or rather a neoplastic one, it has become evident that an immunological derangement leading to an abundant cytokine production is one of the key mechanisms responsible for the diverse disease manifestations.^{2,3,32–34} In contrast to malignant diseases in which the conditioning regimen is warranted in order to eradicate the patient's hematopoiesis, the therapeutic effect of SCT in LCH may result from the suppression and modulation of a severe immunological dysregulation by both, the highly immunosuppressive conditioning regimen and the donor immune cells.

In our study cohort, the RIC regimen was well tolerated and transplant-related toxicity and mortality was considerably lower than for myeloablative conditioning regimens reported to date. Two deaths occurred among the nine patients included in our analysis, which translates into an overall survival rate of 78% in risk patients, compared to a 48% survival among 29 risk patients with myeloablative SCT reported in the literature (Table 4). One patient, with heterozygote alpha-1-antitrypsin deficiency, liver cirrhosis at diagnosis and a severe LCH-related pulmonary dysfunction, requiring assisted ventilation at the time of SCT, died due to virus (CMV)-induced respiratory distress syndrome.

Apart from this death and another death due to septic complications, all other patients experienced expectable mild-to-moderate transient procedure-related complications only and these patients are alive and in good clinical condition 215–881 days after transplantation with no evidence of AD in six and stable disease activity in one (Table 3). Therefore, it seems probable that the low transplant-associated morbidity and mortality of RIC-SCT may have translated into a significantly improved survival for this high-risk patient population. However, it has to be stressed that the median observation time after SCT is still too short to conclude on the long-term course of the disease itself.

Overall, our data, together with the data published by Rao *et al*³⁵ on two patients, who underwent successful RIC-SCT for high-risk LCH, demonstrate that RIC-SCT is a feasible treatment option for high-risk LCH and that cure can be achieved without myeloablation. Notably, even in those patients who responded well to RIC-SCT, clinical recovery from the underlying disease after transplantation was slow and protracted (Figure 1). This might reflect a slow, gradual decrease of cytokine load and related symptoms and seems to be a peculiarity for this disease, which the transplanting physician should be aware of.

Lineage-specific chimerism seems to have an important impact on the outcome following reduced-intensity conditioning concerning graft rejection.³⁶ The impact of donor chimerism within the different cell populations on LCH remains open. The therapeutic effect of allogeneic SCT

Table 4 Literature review: outcome of pediatric LCH patients with risk organ involvement, who underwent allogeneic SCT

| Reference | Age at SCT (months) | Donor | Conditioning | Outcome | Survival (days after SCT) | Cause of death |
|--|---------------------|-------------------|-------------------------------------|-----------------|---------------------------|---|
| Greinx <i>et al</i> ¹¹ (UPN5187) | 25 | MSD | TBI/CY | Alive, NAD | > 820 | — |
| Frost and Wiersma ¹² | 16 | MSD | TBI/CY/VP16 | Alive, NAD | 480 | — |
| Conter <i>et al</i> ¹³ | 27 | MSD | BU/CY/MEL | Alive, NAD | 750 | — |
| Broadbent and Ladisch ^{14a} (pt. 1) | 20 | MSD | TBI/CY/VP16 | Dead | Unknown | Transplant-related (VOD liver) |
| Broadbent (pt. 2) | 22 | MSD | BU/CY/VP16 | Dead | Unknown | Transplant-related (respiratory failure) |
| Broadbent (pt. 3) | 12 | Haplo (2x) | 1. BU/CY/VP16/ATG 2. TBI/MEL/ATG | Dead | Unknown | Transplant-related (capillary leakage) |
| Broadbent (pt. 4) | 20 | MSD | TBI/CY/VP16 | Dead | Unknown | LCH-related (progressive disease) |
| Broadbent (pt. 5) | 15 | MSD | BU/CY | Alive, with LCH | Unknown | — |
| Broadbent (pt. 6) | 65 | MSD | BU/CY/VP16 | Alive, NAD | Unknown | — |
| Broadbent (pt. 7) | 29 | MSD | BU/VP16 | Dead | Unknown | Transplant-related (septicemia) |
| Broadbent (pt. 8) | 8 | MSD | BU/CY | Dead | Unknown | Transplant-related (VOD liver) |
| Broadbent (pt. 9) | 28 | MSD | BU/CY | Alive, NAD | Unknown | — |
| Broadbent (pt. 10) | 14 | MSD | BU/CY/VP16 | Dead | Unknown | Transplant-related (respiratory failure) |
| Ayas <i>et al</i> ¹⁶ | Unknown | MSD | BU/CY/VP16 | Alive, NAD | 365 | — |
| Egeler <i>et al</i> ¹⁵ (1) | Unknown | Haplo | TBI/CY/ATG | Dead | 12 | Transplant-related (respiratory failure) |
| Egeler (2) | Unknown | Haplo | TBI/CY/ATG/TT | Dead | 45 | Transplant-related (adenoviral infection) |
| Kinugawa <i>et al</i> ¹⁷ (1) | 51 | Sibling 4/6 | BU/CY/MEL | Alive, NAD | 1020 | — |
| Kinugawa (2) | 59 | MSD | TBI/CY/VP16 | Dead | 100 | Transplant-related (septicemia) |
| Kinugawa (3) | 171 | Syngeneic twin | CY/VP16/ATG | Alive, NAD | 1470 | — |
| Kinugawa (4) | 16 | MSD | TBI/CY | Dead | 9 | Transplant-related (septicemia) |
| Suminoe <i>et al</i> ¹⁸ | 17 | CBT unrelated | TBI/VP16/MEL | Alive, NAD | 365 | — |
| Nagarajan <i>et al</i> ¹⁹ | 21 | CBT unrelated | BU/CY/VP16/ATG | Alive, NAD | 730 | — |
| Hale <i>et al</i> ²⁰ (pt. 1) | 3 | Unrelated 6/6 | TBI/CY/Ara-C | Dead | 33 | Transplant-related (multiorgan failure) |
| Hale (pt. 4) | 21 | Unrelated 5/6 | TBI/CY/Ara-C | Alive, NAD | 1620 | — |
| Akkari <i>et al</i> ²¹ (2106132) | 20 | MSD | BU/VP16 | Alive, NAD | 4380 | — |
| Akkari (2106169) | 14 | MSD | BU/CY/VP16 | Dead | 690 | LCH-related (progressive disease) |
| Akkari (2106143) | 29 | MSD | BU/CY | Dead | 32 | Transplant-related (toxicity) |
| Akkari (1406021) | 19 | MSD | BU/CY | Alive, NAD | 630 | — |
| Akkari (1406244) | 37 | Unrelated matched | TBI/VP16 | Dead | 120 | Transplant-related (multiorgan failure) |

^aBroadbent: Patients from citation as specified and from personal communication.

might be due to the eradication of pathologic Langerhans cells by the donor lymphoid cells (graft *vs* histiocytosis effect). Another mechanism might be the correction of the pathologic immunological crosstalk by the replacement of one or more cell populations involved. In our patient cohort, mixed chimerism in T cells together with complete myeloid donor chimerism as well as donor T-cell chimerism with minimal donor myeloid chimerism was associated with a stable resolution of disease activity.

A potential disadvantage of RIC-SCT is the increased risk of nonengraftment and graft rejection. With regard to our patient cohort, stable engraftment following RIC-SCT may be expected for unmanipulated grafts from matched donors, but seems questionable for T-cell-depleted haplo-identical grafts. However, it has to be stressed that nonengraftment or rejection following RIC-SCT is not a life-threatening event, is associated with complete autologous hematopoietic recovery and, importantly, does not necessarily implicate an exacerbation of LCH. Furthermore, the highly immunosuppressive conditioning regimen together with the pharmacologic GvHD and rejection prophylaxis may decisively contribute to the stabilization of the disease. This has been shown by Akkari *et al*²¹ and Kinugawa *et al*¹⁷ who reported on two patients who failed engraftment followed by a complete autologous recovery of hematopoiesis and resolution of disease activity. Both patients were still alive and disease free 12 and 3 years after transplantation at the time of reporting. A similar clinical course was observed in one of our patients (pt. 2). Retrospectively, it remains open whether the role of the donor immune system, the conditioning regimen, particularly the use of Campath,³⁷ or, if administered, the post transplant immunomodulation (eg etanercept)³⁸ achieved the disease remission in these patients with nonengraftment or rejection following SCT.

In conclusion, RIC-SCT in high-risk LCH is a promising new salvage approach for LCH patients with resistant risk organ involvement. Further studies are warranted in order to evaluate the impact of the immunosuppressive conditioning as well as the lineage-specific chimerism on the outcome following RIC-SCT for high-risk LCH.

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