Aus der

Universitätsklinik für Kinder- und Jugendmedizin Tübingen

Abteilung Kinderheilkunde I mit Poliklinik

(Schwerpunkt: Allgemeine Pädiatrie, Hämatologie und Onkologie)

Stem Cell Transplantation for Relapsed or Refractory Pediatric Solid Tumors

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von

Illhardt, Toni

2021

Dekan: Professor Dr. B. Pichler

1. Berichterstatter: Professor Dr. P. J. Lang
2. Berichterstatter: Professor Dr. W. Vogel

Tag der Disputation: 29.04.2021

Meiner Familie

Table of contents

Zusammenfassung

Therapierefraktäre oder rezidivierende pädiatrische solide Tumore haben trotz aller Fortschritte der letzten Jahrzehnte weiterhin eine schlechte Prognose. Als potenziell kurative therapeutische Ansätze kommen autologe und allogene (haploidente) Stammzelltransplantationen in Kombination mit intensiven Chemotherapien in Frage. Diese Therapieoptionen wurden in der vorliegenden Arbeit am Beispiel von Nephroblastomen und Neuroblastomen retrospektiv untersucht.

Es konnte gezeigt werden, dass beide Verfahren mit niedriger Toxizität und ohne transplantations-assoziierte Mortalität durchgeführt werden können und damit mit deutlich höheren Chancen auf ein Langzeitüberleben im Vergleich zu anderen Therapieoptionen einhergehen.

Insbesondere die haploidente Stammzelltransplantation bietet für Patienten mit schweren, refraktären oder rezidivierenden Verläufen die Möglichkeit einer „Salvage-Therapie“, selbst wenn alle anderen Therapieoptionen (inkl. der autologen Stammzelltransplantation) ausgeschöpft sind. Die niedrige Rate an Nebenwirkungen verbunden mit einem Langzeitüberleben von ca. 25% bietet diesen Patienten mit sonst infauster Prognose eine letzte Chance auf ein tumorfreies Leben. Zudem liefern die Ergebnisse der Studien eine gute Ausgangslage für moderne Therapiekonzepte wie Immun- oder Zelltherapien.

Aufgrund der Seltenheit dieser Tumore sind systematische Untersuchungen mit großen Fallzahlen nicht möglich. Insbesondere für haploidente Stammzelltransplantation bei Neuroblastomen finden sich in der Literatur häufig nur Einzelfallberichte oder sehr kleine Kohorten. Die vorgestellte Studie stellte zum Zeitpunkt ihrer Veröffentlichung die größte systematisch ausgewertete Kohorte zu diesem Thema dar. Beide Studien konnten damit einen Beitrag zur Verbesserung der Prognose dieser schwer therapierbaren Patientengruppen leisten.

Die Ergebnisse dieser Studien lassen den Schluss zu, dass diese Therapiekonzepte im Hinblick auf Machbarkeit, Toxizität und transplantations-assoziierter Mortalität sehr wahrscheinlich auf andere solide Tumore übertragbar

sind und bieten damit eine erfolgsversprechende Alternative zur palliativen Behandlung für alle Kinder mit refraktären oder rezidivierenden soliden Tumoren.

Summary

Refractory or recurrent pediatric solid tumors continue to have a poor prognosis despite all the progress made in recent decades. Autologous and allogeneic (haploidentical) stem cell transplantation in combination with intensive chemotherapy are potentially curative therapeutic approaches. These therapeutic options have been retrospectively investigated in the presented studies using nephroblastoma and neuroblastoma as examples.

It could be shown that both procedures can be performed with low toxicity and without transplantation-related mortality and thereby raising the chances of long-term survival significantly compared to other therapy options.

In particular, haploidentical stem cell transplantation offers the possibility of a "salvage therapy" for patients with severe, refractory, or recurrent disease, even when all other therapy options (including autologous stem cell transplantation) have been exhausted. The low rate of side effects combined with a long-term survival rate of approximately 25% offer these patients with otherwise unfavorable prognosis a last chance for a tumor-free life. Additionally, the results of the studies provide a good starting point for modern therapeutic concepts such as immune or cell therapies.

Due to the rarity of these tumors, systematic studies with large numbers of patients are not possible to conduct. Especially for haploidentical stem cell transplantation in neuroblastomas, the literature often comprises only individual case reports or very small cohorts. At the time of its publication, the presented study comprises the largest systematically evaluated cohort on this topic. Hopefully, both studies thus contributed to improving the prognosis of these difficult to treat patient groups.

The results of these studies suggest that these therapeutic concepts are very likely to be transferable to other solid tumors in terms of feasibility, toxicity, and transplantation-related mortality and thus offer a promising alternative to palliative treatment for all children with refractory or recurrent solid tumors.

Introduction

Over the last 50 years, the prognosis of pediatric malignancies has drastically improved, reaching approximately 85% five-year survival rates in developed countries [1]. Despite this progress, pediatric solid tumors remain more difficult to treat, with lower five-year survival rates than non-solid tumors [1]. This is particularly distinctive in high-risk solid tumors, such as relapsed or refractory nephroblastoma and neuroblastoma [2, 3]. Hence, it is of vital importance to improve current treatment options and develop new strategies for these entities. Stem-cell transplantation (SCT) is an effective treatment option for pediatric leukemias, but it has additionally been proven to be a feasible therapy for solid tumors [4].

This thesis aimed to present two peer-reviewed studies to show the practicability and efficiency of SCT in high-risk nephroblastoma and neuroblastoma. Firstly, general information on nephroblastoma and neuroblastoma are presented. Then, different types of SCT procedures used in the studies are introduced.

Nephroblastoma

A nephroblastoma, also known as Wilms' tumor, is the most common renal malignancy in childhood. It was first described in 1899 by the German surgeon Max Wilms. It is a rare malignancy with an incidence of approximately eight out of one million children [1]. Applying multimodal therapy concepts, five-year survival rates have significantly improved from approximately 70% in 1972 to 96% in 2016 [1]. However, survival rates in high-risk patients are significantly lower due to chemotherapy resistance or metastases, as described in the introduction of Chapter 1 in more detail.

The prognosis of nephroblastoma largely depends on the following factors:

- Stage at initial diagnosis
- Histopathology of the tumor (favorable vs. anaplastic histology)
- Molecular features of the tumor
- Age at initial diagnosis (older age is associated with poor prognosis)

Staging is generally carried out according to the guidelines of the National Wilms Tumor Study Group (NWTS)[5]:

- Stage 1: Tumor limited to one kidney; tumor capsule intact, complete resection possible
- Stage 2: Tumor spreads beyond the kidney capsule (fat, connective tissue, blood vessels), complete resection possible
- Stage 3: Tumor has spread to adjacent organs or lymph nodes but has not left the abdominal cavity. A complete resection is no longer possible
- Stage 4: Tumor has spread to distant organs such as bones, lungs, brain, or lymph nodes outside the abdomen
- Stage 5: bilateral Wilms' tumor

All stages at initial diagnosis can be approached with conventional chemotherapy and surgery.

However, this staging procedure only applies to the initial diagnosis or responsive nephroblastoma. With respect to refractory or recurrent nephroblastoma, the prognosis is significantly worse, and no cure can be expected solely using conventional therapy methods. Therefore, in Chapter 1, the investigation of an alternative treatment option for refractory or recurrent nephroblastoma with autologous SCT is presented.

Chapter 1 therefore introduces the feasibility of treating refractory or recurrent nephroblastoma with autologous SCT since the prognosis for these cases is significantly worse, and no cure can be expected with conventional therapy methods alone.

Neuroblastoma

The term *neuroblastoma* describes a variety of solid tumors that are derived from sympathetic ganglion cells. It is the most common cancer in infants and accounts for 4–6% of all cancer entities in childhood. In 90% of cases, neuroblastoma is diagnosed before the age of five. Patients older than 10 years of age at initial diagnosis are rare [6].

The tumor location of the primary tumor varies according to the derivation from the sympathetic cells. The most common location is the adrenal gland (being accountable for the most common first sign – a swollen mass in the belly). Other less common locations are the abdominal, thoracic, and cervical sympathetic nodes.

At the initial diagnosis, two-thirds of neuroblastomas are already metastasized in the (local) lymph nodes, bones, bone marrow, or lung. The primary staging consists of an ultrasound, whole-body imaging (CT-Scan or MRI), MIBG-Scan, bone marrow aspiration (to exclude bone marrow metastases), tumor biopsy, and urine examination for the catecholamine breakdown products homovanillic acid and vanillylmandelic acid (since a majority of neuroblastomas produce catecholamines). The prognosis depends on a number of features such as the following:

- Age at initial diagnosis
- Tumor size
- Presence of metastases
- Tumor histology
- Tumor genetics (particularly the MYCN-oncogene being an unfavorable parameter if present) including changes in chromosomes and the total number of chromosomes
- Blood parameters (e.g. neuron-specific enolase, ferritin, and lactate dehydrogenase).

Accordingly, neuroblastomas can be divided into four different risk groups: very-low risk, low risk, intermediate risk, and high-risk tumors. The prognosis depends on the corresponding risk group, reaching approximately >85% for very low, >75-85% for low, 50–75% for intermediate, and <50% for high-risk tumors [7].The prognosis is even worse in refractory (non-responsive to initial treatment) and relapsed tumors. Therefore, novel treatment options for these patients are crucial.

Autologous Stem Cell Transplantation and High-Dose Chemotherapy

In patients with difficult to treat cancer entities, oncologists face a dilemma. They desire the use of the most efficient chemotherapy, which is generally identical to the most toxic treatment. After significantly high doses of chemotherapy with or without radiation therapy, the bone marrow of the patient is occasionally damaged beyond repair or the recovery takes an excessive amount of time, tremendously increasing the risks (e.g. infections). This high-dose chemotherapy is occasionally referred to as *myeloablative chemotherapy* due to the annihilating effect on the stem cells.

In order to boost the recovery of the bone marrow after such a therapy, the autologous SCT is applied since the procedure provides a feasible way to use extremely high doses of chemotherapy and to simultaneously deal with the most severe side effects. As a result, stem cells from the patients are isolated before (myeloablative) chemotherapy is conducted. Normally, there are two different approaches to extract and collect stem cells: from peripheral blood or via direct bone marrow aspiration.

For peripheral blood extraction, distinct drugs (e.g. granulocyte-colony stimulating factor [G-CSF]) are used to stimulate stem-cell production and mobilization from the bone marrow into the blood stream. After mobilization, these cells can be filtered from the peripheral blood via apheresis until a sufficient amount of stem cells are collected.

The second method is the direct extraction of stem cells from the bone marrow via bone marrow biopsy. This is generally conducted from the iliac crest. Then, the collected stem cells are purified and frozen at -80° to -160 C. Under these conditions, cells can be used indefinitely. After conduction of high-dose chemotherapy, it generally takes 36–48 hours following the last chemotherapy cycle until drug levels drop to a non-ablative level. After this, the collected stem cells can be transfused into the peripheral blood of the patient where they will

autonomously proliferate in the bone marrow and produce all three types of bone-marrow-derived blood cells: leukocytes, erythrocytes, and thrombocytes.
The entire procedure of stem cell collection, myeloablative chemotherapy, and stem cell transfusion is occasionally referred to as “autologous stem cell rescue”, as stem cells rescue the bone marrow from the effects of the high-dose chemotherapy. This procedure is generally performed once per patient but can be administered twice if necessary.

The efficiency of high-dose chemotherapy with autologous stem-cell rescue has been proven to be beneficial in different high-risk pediatric solid tumors, for example in neuroblastoma, nephroblastoma, sarcoma, medulloblastoma, germ cell tumors, and rhabdomyosarcoma, with tolerable side effects and low treatment related mortality [4, 8]. Survival rates vary significantly depending on the underlying disease, risk classification, intensity of previous therapies, and other factors.

Haploidentical Stem Cell Transplantation

Haploidentical SCT is a specific procedure of allogeneic SCT. Allogeneic (in contrast to autologous) means that the stem-cell recipient and donor are genetically different. The greater the genetic variability between the donor and the recipient, the higher the risk of alloimmune side effects. This includes the risk of transplant rejection, slow stem-cell recovery, and graft-versus-host disease (GvHD), a major complication of allogeneic stem-cell transplantation. The key factors to genetic similarity in terms of SCT are the human-leukocyte antigens (HLA). HLAs are a heterogenous group of proteins represented in nearly all human cells and tissues. They significantly vary between individuals of the same species and act like a form of genetic fingerprint. The immune system uses this mechanism to distinguish between host and foreign cells. For example, due to the changes the virus causes to the cell DNA, infected cells tend to change their HLA antigens. Subsequently, these infected cells can be specifically targeted and destroyed by leucocytes without harming healthy cells.

This mechanism is advantageous in healthy individuals but disadvantageous when allogeneic SCT is required. Due to differences in HLA antigens, the transplanted cells are immediately recognized as foreign and destroyed (leading to transplant rejection) or the newly derived immune system attacks the recipient's tissue (leading to GvHD).

As previously stated, the likelihood of these scenarios changes with the degree of variation in HLA antigens. Therefore, a donor with the exact same type of antigens would be excellent. Since HLA antigens are inherited randomly from the father and mother to the children and have a high degree of genetic variability, HLA-identical donors are rare. Even among family members, only identical twins are generally HLA-identical. The search for a suitable donor can, therefore, take a long time via international databases, and for certain ethnic groups, it is nearly impossible to find a matching donor due to rare antigen constellations or low numbers of registered donors of a specific ethnic group.

To circumvent this problem, the procedure of haploidentical SCT was developed. Here, a donor is sought in which at least half of the HLA antigens are identical. Since children inherit half of the HLA antigens of each parent, both parents and all siblings are haploidentical and suitable as potential donors for the (respective) patient. Compared to autologous or fully identical allogeneic transplantation, the risk of rejection or of GvHD is increased. However, modern myeloablative chemotherapy regimens and drug-based GvHD prophylaxis have significantly reduced the risk in recent years, resulting in low rates of rejection, chronic or acute GvHD, and transplant related mortality [9-11].

The process of stem cell collection is equal to that of autologous transplantation (see above).
Generally, a haploidentical transplantation is performed after an (unsuccessful) autologous transplantation has already taken place. Apart from the option of an additional chemotherapy, immunological processes are described for allogeneic (e.g. haploidentical) transplantation, where the newly established immune system

is directed against tumor cells. This effect is additionally known as the *graft-vs-tumor* or *graft-vs-leukemia* (in non-solid tumors) effect and can have a beneficial impact on the outcome [12-14].

Haploidentical SCT has been successfully used to treat several solid pediatric tumors, including neuroblastoma, nephroblastoma, rhabdomyosarcoma, and Ewing's sarcoma [9, 11, 14]. However, a majority of these studies have lacked a sufficient number of patients, and the role of haploidentical SCT in solid tumors has not been systematically investigated. Due to the rarity of these diseases, large and controlled randomized studies will not be possible to be conducted in the foreseeable future.

We present data from the largest published cohort on haploidentical SCT for the treatment of neuroblastoma at the time of publication and hope to contribute to the care of this special patient group. This applies to the specific therapy of high-risk neuroblastomas as well as to the application of haploidentical transplantation in solid tumors in general.

The aim of this work was to further contribute to the evaluation of this procedure in high-risk patients and to show that this approach is feasible with few side effects and promising prognosis.

Chapter 1

Children with Relapsed or Refractory Nephroblastoma: Favorable Long-term Survival after High-dose Chemotherapy and Autologous Stem Cell Transplantation

T. Illhardt[1]*, M. Ebinger[1]*, C. P. Schwarze[1], T. Feuchtinger[1], R. Furtwängler[2], P. G. Schlegel[3], T. Klingebiel[4], J. Greil[5], J. F. Beck[6], R. Handgretinger[1], P. Lang[1]

* T. Illhardt and M. Ebinger contributed to this work equally and share first authorship

Affiliations
[1] Department of Pediatric Oncology, University Children's Hospital, Tuebingen, Germany
[2] Department of Pediatric Oncology, University of Saarland, Homburg,Germany
[3] Departments of Pediatric Hematology and Oncology, and Stem Cell Transplantation Program, University Children's Hospital, University of Wuerzburg, Wuerzburg, Germany
[4] Department of Pediatric Oncology, University Hospital Frankfurt, University of Frankfurt, Frankfurt, Germany
[5] Pediatric Oncology and Hematology, University Hospital Heidelberg, Heidelberg, Germany
[6] Department of Pediatric Hematology and Oncology, University Hospital Jena, Jena, Germany

Klin Padiatr. 2014 Nov;226(6-7):351-6. doi: 10.1055/s-0034-1390504. Epub 2014 Nov 28.

ABSTRACT

Background

High-dose chemotherapy (HDC) with autologous stem-cell rescue (ASCR) is a treatment option for pediatric patients with relapsed nephroblastoma. We present long term results of 9 patients treated between 1993 and 2013 at our center.

Procedure

Reinduction therapy was carried out according to GPOH and SIOP recommendations. The conditioning regimen consisted of Carboplatin (1200mg/m²), Etoposide (800mg/m² or 40mg/kg) and Melphalan (180mg/m²). Purging of the grafts with immunomagnetic CD34 positive selection was performed in 5 patients.

Results

8 of 9 Patients (90%) are alive without evidence of disease after a median follow-up of 8.5 years. Leukocyte engraftment occurred after a median of 10 days (range 8-12). Median numbers of 667/µl CD3+, 329/µl CD4+, 369/µl CD8+ T cells and 949/µl B cells were reached after 180 days. No negative impact of CD34 selection was observed. No transplantation-related death occurred. Acute toxicity comprised mucositis III°-IV° in all and veno-occlusive disease in one patient. Long term effects probably related to treatment occurred in 3/7 evaluable patients and comprised hearing impairment, reduced renal phosphate reabsorption, mild creatinine elevation and hypothyroidism (n=1, each).

Conclusion

Thus, in our experience HDC with ASCR is an effective treatment of recurrent or refractory Nephroblastoma with acceptable side effects. However, a randomized trial proving its efficiency with a high level of evidence is needed.

INTRODUCTION

In the SIOP93-01/GPOH (International Society of Paediatric Oncology/Society for Paediatric Oncology and Haematology) trial 9 % of all patients with a localized nephroblastoma suffered from recurrence [18]. This rate is even higher in patients

with one or more risk factors including unfavorable/high risk histology, older patients, bilateral disease and high tumor stage. Metastatic disease at diagnosis (Stage IV) for example reduces the EFS to 72 % in GPOH patients [20]. Fortunately, the outcome of patients with recurrent disease has greatly improved over the last 30 years. While the overall survival before the mid-1980s hardly reached 20–30 % [7, 8, 17, 23], current treatment regimens are able to accomplish survival rates between 45–60 % for relapsed patients [1, 4, 13, 14, 22]. Especially the introduction of carboplatin, etoposide and ifosfamide in the late 80s and early 90s resulted in a major improvement in survival rates [16]. At the moment 3-year overall survival for standard risk relapse reaches 75–85 % [5]. In contrast, high and very high-risk relapse patients have a significantly lower survival (50–60 % and 15–25 %) [5, 19] and might potentially profit from increased treatment intensity. The significance of high-dose chemotherapy (HDC) in connection with autologous stem-cell rescue (ASCR) remains undetermined. As stated by previous studies there is evidence for this method to be very effective in treating relapsed nephroblastoma [3, 5, 6, 11, 12, 15, 19]. Promising 3- to 5-year survival rates between 30 % and 60 % have been reported. Recently a retrospective analysis from the GPOH compared 26 matched cases receiving and not receiving HDC and ASCR resulting in 64 % vs. 45 % 3 year-overall survival ($p = 0.059$) [5]. However, the number of cases in these studies is small and due to the small number of patients with high risk relapsed nephroblastoma in general, randomized studies are hard to perform. Thus, more research on this topic seems to be necessary. The purpose of this paper is to report the experience with HDC and ASCR in terms of survival, side effects and long-term toxicity.

PATIENTS AND METHODS

Between 1993 and 2013, 9 patients with relapsed or primary refractory nephroblastoma were enrolled in this retrospective monocenter analysis. All of them were treated with HDC and ASCR. Informed consent was obtained from the legal guardians and patients. Table 1 gives an overview about patient characteristics and medical history before HDC. The median age at initial

diagnosis was 53 months (Range 11–111 months). According to the SIOP2001/GPOH trial protocol tumor histology was stratified into 3 risk groups (low-risk, intermediate-risk and high risk). Histological stratification was performed by central pathology review and revealed intermediate-risk tumors in 6 patients and high-risk tumors in 2 patients. The histology of patient # 7 was not determined by reference pathology. 4 patients were diagnosed with stage IV disease, 3 of them with pulmonary metastases and one with multiple metastases in lung, liver and pancreas.

Table 1 Patient characteristics and initial treatment.

ID	Sex	Age at ID (months)	Initial Stage	Histologic risk stratification	Localization Primary Tumor	Initial Metastases	Treatment prior to HDC/ASCR Chemotherapy First/Second/Third†	Surgery	Radiotherapy After surgery	After SCT	Number of Relapses before HDC/ASCR
1	F	111	IV	III	R	P, peritonal seeds (H, Pa), LN	AVD/CpDEC * /ICE	+	–	–	0 (progress)
2	F	59	IV	II	L	P	AVD/ICE	+	–	–	1
3	M	39	IV	II	L	P, LN	AVD/ICE	+	+	–	1
4	F	48	I	II	L	–	AVD/CE/ICE	+	+	+	2
5	F	53	II–III	III	L	–	AV/CE + ID	+	+	–	0 (partial remission)
6	F	46	IV	II	R	P	AVD/ICE	+	–	+	0 (progress)
7	M	103	II	unknown	R	–	AV/ICE	+	–	+	1
8	M	63	II	II	L	–	AV/CE	+	+	–	1
9	M	11	III	II	R	–	AV/CE	+	+	–	1

Explanations: Histology, I = low risk, II = intermediate risk, III = high risk; Metastases: P = pulmonary, H = hepatic, Pa = pancreatic, LN = local lymph nodes; Chemotherapy: A = Actinomycin-D, V = Vincristin, D = Doxorubicin, Cp = Cyclophosphamide, E = Etoposide, C = Carboplatin, I = Ifosfamide, Surgery: L = local, M = metastases
* more intense regimen due to tumor progress post-operative
†last chemotherapy performed corresponds to "salvage therapy"

Initial Therapy

All patients were treated with preoperative chemotherapy. Pre- and postoperative chemotherapy included actinomycin D and vincristine in 3 patients, 5 patients additionally received doxorubicin. Due to tumor progression under chemotherapy, postoperative treatment of patient #1 was changed to the high-risk arm (cyclophosphamide, doxorubicin, etoposide and carboplatin) after surgery. In all other patients postoperative chemotherapy consisted of additional courses of the initial treatment. 5 Patients received local radiotherapy with 15 Gy cumulative dose after surgery (Table 1).

Relapse

Table 2 gives an overview about the time to relapse and the site of relapse. 6 patients relapsed after a median of 8 months (Range 6–14 months). Progression under primary therapy occurred in 2 patients and one patient showed only partial remission under initial treatment. One patient (# 4) experienced 2 relapses before HDC. The first local recurrence was treated with an additional surgical intervention in combination with standard chemotherapy (carboplatin + etoposide) and local irradiation.

This second relapse with metastases in liver and lung (after 5 months) was treated with salvage therapy (ifosfamide, carboplatin and etoposide), HDC and ASCR. Most relapses occurred in the lung (6 patients), followed by liver (2 patients) and CNS (2 Patients).

Salvage chemotherapy consisted of ifosfamide, carboplatin and etoposide (ICE) in 6 patients according to the recommendation of the SIOP-GPOH, one patient received doxorubicin in addition to ICE and 3 patients were treated with carboplatin and etoposide only (Table 2).

Table 2 Relapse, HDC parameters and outcome.

ID	Time to Relapse	Site of Relapse	Status before HDC/ASCR	Infused CD34+ cells $\times 10^6$ per kg BW	CD34-Enrichement	RT after HDC/ASCR	Outcome	Follow-up Interval (months)
1	–	–	progression	7.89	–	–	DOD	0.7
2	9	P	CR 2	16.84	+	–	NED	92
3	14	P	CR 2	7.06	–	–	NED	7.7
4	8	1. local 2. P/H	CR 3	10.2	–	+	NED	124
5	0*	H	CR1	18	+	–	NED	127
6	–	CNS, P	progression	14.41	+	+	NED	102
7	14	P	PR	1.91	–	+	NED	101
8	6	Sp	CR 2	1	+	–	NED	189
9	8	P	CR 2	15.4	+	–	NED	109

Explanations: CR = Complete Remission; PR = Partial remission; HDC = high-dose chemotherapy; P = pulmonary; H = hepatic; Sp = spinal; RT = Radiotherapy; DOD = died of disease; NED = no evidence of disease

* hepatic metastases occurred directly after initial treatment

HDC and ASCR

HDC was considered as treatment for patients with high risk relapse according to the recommendation of the SIOP-GPOH 2001 trial or for primary refractory/progressive patients. The criteria for high risk relapsed nephroblastoma are shown in Table 3.

All patients met at least one criterion. HDC was performed after salvage therapy. Remission status before HDC is shown in Table 2. 6 patients had complete remission, 1 patient showed partial remission and 2 patients had progressive disease prior to HDC. Peripheral stem cells were harvested after mobilization with G-CSF. CD34-enrichment with Magnetic-activated Cell Sorting (MACS) was performed between 1994 and 2005 in 5 patients as seen in Table 2. After 2005, unmanipulated peripheral stem cells were used for transplantation. The conditioning therapy consisted of a 3-drug regimen containing carboplatin (400 mg/m²/d over 3 days), etoposide (800 mg/m² or 40 mg/kg BW on one day) and melphalan (45 mg/m²/d over 4 days) (CEM). The target dose for carboplatin was adjusted according to GFR monitoring: 1. For patients with prior nephrotoxic

therapy: calculated target dose in percent (%CTD) = (0.65 × GFR) + 18. 2. For patients without prior nephrotoxic therapy: %CTD = (0.82 × GFR) + 18. Adjuvant radiotherapy was performed in 3 patients with a cumulative dose of 15 Gy to the relapse tumor bed.

Table 3 Definition of high-risk relapsed nephroblastoma according to the SIOP-GPOH 2001 protocol.

Criteria
– Initial Stage III or IV
– ≥3 Agents in Initial Treatment
– High-risk Histology (anaplastic or blastema-rich)
– Uncommon Relapse Sites (CNS, Bone)
– Early Relapse (<6 months)
– Relapse in Different Organs or Multiple Metastases in Both Lungs (>5)
– Lymph Node Metastases (esp. mediastinal or inguinal)
– Relapse in Previously Irradiated Areas

Statistics

Event-free survival (EFS) and overall survival were estimated by Kaplan-Meyer-Method. Data analysis was performed using SPSS® (Version 20).

Follow-up

Regular follow-up examination was performed 1, 2, 3, 6, 12, 18, 24, 36, 48, 60, 84 and 120 months after transplantation. Routinely accomplished investigations included blood samples, ultrasound scans of the abdomen and the thyroid gland, bone marrow biopsies, spirometries, ECG, echocardiography, ophthalmologic and otorhinolaryngologic examination.

RESULTS

Acute Toxicity

Acute toxicity after stem-cell transplantation was classified according to adapted National Cancer Institute Common Terminology Criteria 3.0. No transplant related mortality occurred. Acute side effects of the conditioning regimen consisted of mucositis III °–IV ° requiring continuous morphine therapy as well as parenteral nutrition in all patients. 5 patients developed diarrhea.

Nausea was observed in 5 patients. Fever during aplasia occurred in 8 patients without identification of infectious agents. Transient renal involvement with elevated creatinine (> 3.0 × upper limit of normal [ULN]) and also transient elevated levels of bilirubin (> 3.0 × ULN), AST and ALT (> 5.0 × ULN) occurred in 7 and in 4 patients, respectively.

One patient (ID1) developed hepatic veno-occlusive disease (VOD) with portal flow reversal under conditioning therapy. Multiple hepatic metastases in this patient might have increased the risk. Under the treatment with defibrotide the VOD resolved. Unfortunately, this patient died a few weeks later due to tumor progression.

No cardiac, pulmonary or neurological side effects were observed.

Engraftment

A median number of 10.2 × 106 CD34 + stem cells per kg BW were infused (Range 1–16.84) as seen in Table 2. All patients received G-CSF after transplantation. Absolute leukocyte count > 1 000/µl (ALC) and absolute neutrophile count > 500/µl (ANC) were reached after a median time of 10 days (Range: ALC 8–12, ANC 8–15). Flow cytometry analyses were performed on day 14, 21, 30, 60, 90, 180 and 365 after ASCR and later on after variable intervals. Reconstitution of lymphocyte subsets over the first year after ASCR is shown in Fig. 1. Median T-Lymphocytes (CD3) increased from 320/µl (Day 30, Range 69–1 354) to 667/µl (Day 180, Range 106–1 658). Normal values were reached after 1 year (> 1 000/µl). Median Cytotoxic T cells (CD8) increased from 130/µl (Day 30, 40–1 023) to 369/µl (Day 180, Range 150– 698). Median T helper cells increased from 240/µl (Day 30, Range 19–280) to 329/µl (Day 180, Range 150–698). Normalization was reached after one year (> 500/µl). Median B-lymphocytes (CD 19) increased from 172/µl (Day 30, Range 1–735) to 949 (Day 180, Range 329–1 375). These elevated levels normalized after 3 years (not shown).

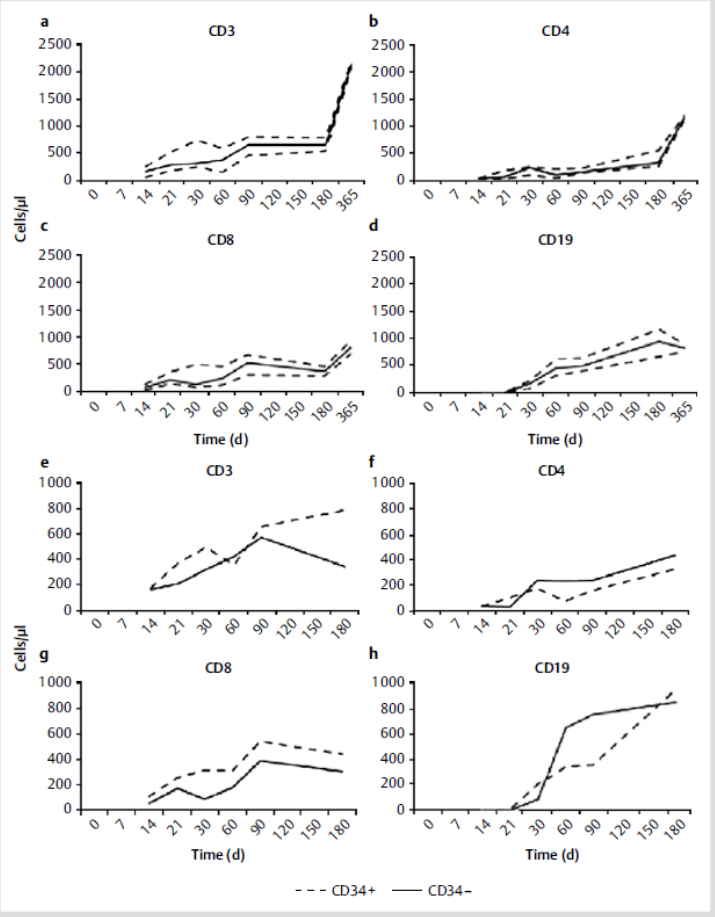

Fig. 1 Shown are the median developments of CD3+, CD3+4+, CD3+8+ T cells and CD19+ B cells. Figures **a–d** show the median reconstitution over a one year period in all patients. The 25% quantile and the 75% quantile are shown dashed. T-lymphocytes normalized after approximately one year. Figures **e–h** show a comparison of median T- and B- cell recovery in CD34-purged patients and non-purged patients over a 180 day follow-up period. CD-34 purged patients are shown drawn trough (–) and nonpurged patients are shown dashed (---). CD34+ selection was used in 5 patients between 1994 and 2005. Descriptive analyses shows no distinct difference between purged and non-purged patients. No statisticial analysis could be performed in this small number of patients.

Long term toxicity

Long term toxicity effects after a median follow-up of 8.5 years were observed in 3 of 7 evaluable patients. Patient #1 was excluded due to early death and very short follow-up period. Patient #7 has been lost to follow-up. One patient suffered from hearing impairment for high frequencies, mitral regurgitation, lowered renal phosphate re-absorption and elevated levels of estrogens.

One patient showed mild elevation of creatinine (0.9 mg/dl, ULN 0.8 mg/dl) and one patient was diagnosed with hypothyroidism during this follow-up period. The role of chemotherapy in the development of these late side effects remains undetermined. Incidental findings should be considered. In all other patients there were no signs of long-term toxicity.

Survival

Event-free survival was defined as relapse-free or progression free time after transplantation. 8 of 9 patients (90 %) are alive with no evidence of disease after a median follow-up of 8.4 years (Range 0.06–15.5). Only one patient with progressive disease prior to transplant died during salvage therapy 3 weeks after HDC due to tumor progress. On the contrary the outcome of another patient (# 6) with progressive disease before transplantation was favorable. This patient received local irradiation in addition to HDC and is alive without evidence of disease after 8.4 years of follow-up. The patient with partial response (ID 7) was also irradiated for local tumor control.

DISCUSSION

The significance of high-dose chemotherapy (HDC) in combination with autologous stem-cell rescue (ASCR) still remains unclear in relapsed nephroblastoma since randomized studies with a high number of cases are lacking. However, several non-randomized studies have shown favorable overall survival and event-free survival rates between 34–64 % (13–19) and HDC with ASCR therefore seems to be a very effective way to treat relapsed nephroblastoma. We could confirm these results in our retrospective, monocenter analysis with a long term EFS of 90 % after a median follow-up of 8.4 years. Since 7 patients are in complete remission after a follow-up of more than 7 years, recurrence in these patients is most unlikely. However, our results are limited by several factors of influence: the number of cases was low, the initial therapy was not standardized and a heterogeneous group of patients had to be recruited for analysis.

With these results, we cannot definitely answer the question whether HDC therapy is superior to a non-HDC approach or not. A recent meta-analysis by Ha et al. [9] pointed out that further data on this topic is needed, especially for the rarer subgroups, i. e., high-risk histology. However, this meta-analysis showed that HDC might be more effective in terms of both EFS and OS, compared to non-HDC treatment. The authors pointed out that especially patients in the high

risk (every patient with initial RT, ≥ 3 drugs during initial treatment, initial use of ifosfamide or cyclophosphamide) or very high-risk subgroups (stage IV disease with unfavorable histology, initial therapy with 4 or more chemotherapeutic agents, > 1 relapse or refractory relapse) seem to benefit from HDC treatment with improved EFS. The calculated hazard ratios were 0.97 (95 % CI 0.43–2.71, p = 0.94), 0.90 (95 % CI 0.62–1.31, p = 0.6) and 0.50 (95 % CI 0.31–0.82. p = 0.006) for the standard, high and very high-risk subgroups respectively as compared to non-HDC treatment. This suggests a significant benefit for the very high-risk subgroup and a potential benefit for the high-risk group, whereas the benefit for the low risk group seems negligible. The estimated 3-year EFS for standard, high and very high risk were 59.5 %, 49 % and 27.2 % respectively over all relapsed patients (treated with or without HDC). In conclusion the potential benefit of HDC therapy in comparison to Non-HDC treatment is directly correlated to the individual risk profile of the patient. All but one patient (#7) of our analysis met their criteria for high or very high-risk relapse. These criteria do not coincide with the risk stratification by the SIOP/GPOH normally used for high risk nephroblastoma (Table 3).

On the other hand, possible side effects of HDC must be taken into consideration. Myeloablative regimens tend to increase the possibility of critical side effects like infections or veno-occlusive disease. Therefore treatment-related mortality might be higher than in conventional regimens [21]. However, conflicting results are reported regarding this issue, since a recent metaanalyses performed by Yalcin et al. could not show any significant increase in treatment related mortality, severe infections and sepsis between myeloablative approaches and conventional approaches in the treatment of high-risk neuroblastoma. Nevertheless, they found a higher incidence of renal effects, interstitial pneumonitis and VOD in the myeloablative group [24].

In our analysis no treatment related death occurred. One patient developed VOD which might be at least partially triggered by hepatic metastases. All other patients had only mild and reversible acute toxicities. These results are in line with observations from other investigators and the CEM-Regimen in particular has been considered to be well tolerated with low therapy related mortality [2].

Long term toxicity after HDC/ASCR is not well evaluated and studies about this topic are very rare. Moreover, the contribution of HDC in the development of long-term side effects is difficult to determine considering the fact that all patients received different pre-HDC therapies. Possible late effects cannot be differentiated from other late effects caused by the chemotherapeutical agents used for the HDC. This includes chronic renal, pulmonary or cardiac dysfunction, impairment of the visual or auditory systems, changes in the hormonal systems and secondary malignancies. In our experience, long term toxicity was acceptable since only one patient of 6 evaluable patients suffered from serious long-term side effects (hearing impairment, mitral regurgitation, renal dysfunction and elevated estrogens).

CD34-enrichement was used in 5 patients. The purpose was to deplete the graft of any remaining nephroblastoma cells in order to reduce relapse probability. As shown by Handgretinger et al. [10] CD34 purging is an effective way to reduce graft contamination without hampering the immune recovery. Although this study was performed on patients with high-risk neuroblastoma, we assume that the conclusions can be transferred to nephroblastoma patients. As depicted in Fig. 1, the reconstitution of the observed subtypes does not show any obvious difference between CD34-purged patients and non-purged patients. Due to the low patient numbers, we were not able to demonstrate positive effects on the relapse probability due to CD34 selection in this analysis and probably no large randomized study will have the statistical power to clarify this issue. However, we have not observed any detrimental side-effects like severe infections or delayed immune reconstitution in our patients with CD34 selected grafts. Although the clinical benefit remains unclear, CD34-enrichment may be feasible to reduce tumor cells in the transplant without increasing the side effects.

CONCLUSION

In our experience HDC with ASCR is an effective way to treat recurrent or refractory nephroblastoma and should be considered as the primary relapse therapy for patients under very high risk. An excellent long-term survival without evidence of recurrent disease and with few side effects could be observed.

However, the number of patients analyzed here is small and further investigations on this topic are needed. We suggest a large, standardized, preferably international, randomized trial to determine the role of HDC in terms of survival and toxicity in relapsed and refractory nephroblastoma.

CONTRIBUTOR'S STATEMENT

All authors have contributed to this work in significant ways and agreed upon the content.

Conflict of interest: The authors have no conflict of interest to disclose.

REFERENCES

1. Abu-Ghosh AM, Krailo MD, Goldman SC et al. Ifosfamide, carboplatin and etoposide in children with poor-risk relapsed Wilms' tumor: a Children's Cancer Group report. Annals of oncology: official journal of the European Society for Medical Oncology/ESMO 2002; 13: 460-469
2. Benedetti Panici P, Pierelli L, Scambia G et al. High-dose carboplatin, etoposide and melphalan (CEM) with peripheral blood progenitor cell support as late intensification for high-risk cancer: non-haematological, haematological toxicities and role of growth factor administration. British journal of cancer 1997; 75: 1205-1212
3. Campbell AD, Cohn SL, Reynolds M et al. Treatment of relapsed Wilms' tumor with high-dose therapy and autologous hematopoietic stem-cell rescue: the experience at Children's Memorial Hospital. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 2004; 22: 2885-2890
4. Dome JS, Liu T, Krasin M et al. Improved survival for patients with recurrent Wilms tumor: the experience at St. Jude Children's Research Hospital. Journal of pediatric hematology/oncology 2002; 24: 192-198
5. Furtwängler R, Nourkami N, Alkassar M et al. Update on relapses in unilateral nephroblastoma registered in 3 consecutive SIOP/GPOH studies - A report from the GPOH-nephroblastoma study group. Klinische Padiatrie 2011; 223: 113-119

6. Garaventa A, Hartmann O, Bernard JL et al. Autologous bone marrow transplantation for pediatric Wilms' tumor: the experience of the European Bone Marrow Transplantation Solid Tumor Registry. Medical and pediatric oncology 1994; 22: 11-14
7. Groot-Loonen JJ, Pinkerton CR, Morris-Jones PH et al. How curable is relapsed Wilms' tumour? The United Kingdom Children's Cancer Study Group. Archives of disease in childhood 1990; 65: 968-970
8. Grundy P, Breslow N, Green DM et al. Prognostic factors for children with recurrent Wilms' tumor: results from the Second and Third National Wilms' Tumor Study. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 1989; 7: 638-647
9. Ha TC, Spreafico F, Graf N et al. An international strategy to determine the role of high dose therapy in recurrent Wilms' tumour. European journal of cancer 2013; 49: 194-210
10. Handgretinger R, Lang P, Ihm K et al. Isolation and transplantation of highly purified autologous peripheral CD34(+) progenitor cells: purging efficacy, hematopoietic reconstitution and long-term outcome in children with high-risk neuroblastoma. Bone marrow transplantation 2002; 29: 731-736
11. Hempel L, Kremens B, Weirich A et al. High dose consolidation with autologous stem-cell rescue (ASCR) for nephroblastoma initially treated according to the SIOP 9/GPOH trial and study. Klinische Padiatrie 1996; 208: 186-189
12. Kremens B, Gruhn B, Klingebiel T et al. High-dose chemotherapy with autologous stem cell rescue in children with nephroblastoma. Bone marrow transplantation 2002; 30: 893-898
13. Malogolowkin M, Cotton CA, Green DM et al. Treatment of Wilms tumor relapsing after initial treatment with vincristine, actinomycin D, and doxorubicin. A report from the National Wilms Tumor Study Group. Pediatric blood & cancer 2008; 50: 236-241

14. Park ES, Kang HJ, Shin HY et al. Improved survival in patients with recurrent Wilms tumor: the experience of the Seoul National University Children's Hospital. Journal of Korean medical science 2006; 21: 436-440
15. Pein F, Michon J, Valteau-Couanet D et al. High-dose melphalan, etoposide, and carboplatin followed by autologous stem-cell rescue in pediatric high-risk recurrent Wilms' tumor: a French Society of Pediatric Oncology study. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 1998; 16: 3295-3301
16. Pein F, Tournade MF, Zucker JM et al. Etoposide and carboplatin: a highly effective combination in relapsed or refractory Wilms' tumor a phase II study by the French Society of Pediatric Oncology. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 1994; 12: 931-936
17. Pinkerton CR, Groot-Loonen JJ, Morris-Jones PH et al. Response rates in relapsed Wilms' tumor. A need for new effective agents. Cancer 1991; 67: 567-571
18. Reinhard H, Aliani S, Ruebe C et al. Wilms' tumor in adults: Results of the Society of Pediatric Oncology (SIOP) 93-01/Society for Pediatric Oncology and Hematology (GPOH) Study. Journal of Clinical Oncology 2004; 22: 4500-4506
19. Spreafico F, Bisogno G, Collini P et al. Treatment of high-risk relapsed Wilms tumor with dose-intensive chemotherapy, marrow-ablative chemotherapy, and autologous hematopoietic stem cell support: experience by the Italian Association of Pediatric Hematology and Oncology. Pediatric blood & cancer 2008; 51: 23-28
20. Warmann SW, Furtwangler R, Blumenstock G et al. Tumor biology influences the prognosis of nephroblastoma patients with primary pulmonary metastases: results from SIOP 93-01/GPOH and SIOP 2001/ GPOH. Annals of surgery 2011; 254: 155-162
21. Weaver CH, Schwartzberg LS, Hainsworth J et al. Treatment-related mortality in 1000 consecutive patients receiving high-dose chemotherapy

and peripheral blood progenitor cell transplantation in community cancer centers. Bone marrow transplantation 1997; 19: 671-678

22. Weirich A, Ludwig R, Graf N et al. Survival in nephroblastoma treated according to the trial and study SIOP-9/GPOH with respect to relapse and morbidity. Annals of oncology: official journal of the European Society for Medical Oncology/ESMO 2004; 15: 808-820
23. Wilimas JA, Champion J, Douglass EC et al. Relapsed Wilms' tumor. Factors affecting survival and cure. American journal of clinical oncology 1985; 8: 324-328
24. Yalcin B, Kremer LC, Caron HN et al. High-dose chemotherapy and autologous haematopoietic stem cell rescue for children with high-risk neuroblastoma. The Cochrane database of systematic reviews 2013; 8: CD006301

Chapter 2

Haploidentical Stem Cell Transplantation for Refractory/Relapsed Neuroblastoma

Toni Illhardt [1,†], Jacek Toporski [2,†], Tobias Feuchtinger [1,3], Dominik Turkiewicz [2], Heiko-Manuel Teltschik [1], Martin Ebinger [1], Carl-Philipp Schwarze [1], Ursula Holzer [1], Holger N. Lode [4], Michael H. Albert [3], Bernd Gruhn [5], Christian Urban [6], Josefina H. Dykes [7,8], Oliver Teuffel [1,9], Michael Schumm [1], Rupert Handgretinger [1,‡], Peter Lang [1,‡,*]

[*] T. I. and J.T. contributed equally.
[‡] R.H. and P.L. share senior authorship.

Affiliations
[1] Department of Pediatrics, Children's University Hospital, University of Tuebingen, Tübingen, Germany
[2] Department of Pediatrics, Skåne University Hospital, Lund, Sweden
[3] Department of Pediatrics, Dr. von Hauner Children's Hospital, University Children's Hospital, Munich, Germany
[4] Department of Pediatrics, Children's University Hospital, University of Greifswald, Greifswald, Germany
[5] Department of Pediatrics, Jena University Hospital, Jena, Germany
[6] Department of Pediatrics, Children's University Hospital, Medical University of Graz, Graz, Austria
[7] Department of Clinical Immunology and Transfusion Medicine, Office for Medical Services, Region Skåne, Lund, Sweden
[8] Department of Laboratory Medicine, Lund University, Lund, Sweden
[9] Medical Service of the Statutory Health Fund, Baden-Wuerttemberg, Germany

Biol Blood Marrow Transplant. 2018 May;24(5):1005-1012. doi: 10.1016/j.bbmt.2017.12.805. Epub 2018 Jan 4.

ABSTRACT

Pediatric patients with refractory or relapsed metastatic neuroblastoma (NBL) have a poor prognosis despite autologous stem cell transplantation (SCT). Allogeneic SCT from a haploidentical donor has a remarkable alloreactive effect in patients with leukemia; thus, we evaluated this approach in children with very high-risk NBL. We analyzed data from 2 prospective phase I/II trials. A total of 26 patients with refractory (n = 5), metastatic relapsed (n = 20), or locally relapsed MYCN-positive (n = 1) NBL received a median of 17 × 106/kg T/B cell-depleted CD34+ stem cells with 68 × 103/kg residual T cells and 107 × 106/kg natural killer cells. The conditioning regimen comprised melphalan, fludarabine, thiotepa, OKT3, and a short course of mycophenolate mofetil post-transplantation. Engraftment occurred in 96% of the patients. Event-free survival and overall survival at 5 years were 19% and 23%, respectively. No transplantation-related mortality was observed, and the single death was due to progression/subsequent relapse. The median duration of follow-up was 8.1 years. Patients in complete remission before SCT had a significantly better prognosis than those with residual tumor load ($P < .01$). All patients with progressive disease before SCT relapsed within 1 year. Grade II and grade III acute graft-versus-host disease (GVHD) occurred in 31% and 12% of the patients, respectively. Chronic limited and extensive GVHD occurred in 28% and 10%, respectively. Our data indicate that haploidentical SCT is a feasible treatment option that can induce long-term remission in some patients with NBL with tolerable side effects, and may enable the development of further post-transplantation therapeutic strategies based on the donor-derived immune system.

INTRODUCTION

Despite advances in chemotherapy, surgery, and radiotherapy, patients with refractory or relapsed metastatic neuroblastoma (NBL) continue to have a poor prognosis [1-3]. A recent meta-analysis showed a 5-year survival rate of 15% for second-line chemotherapy followed by high-dose chemotherapy with autologous stem cell rescue, compared with only 4% for nonmyeloablative second-line chemotherapy and 2% for palliative approaches [4]. Several attempts have been

made to evaluate the role of allogeneic SCT in patients with NBL. The graft-versus-leukemia effect is widely acknowledged [5], and there are also indications of a graft-versus tumor (GVT) effect in NBL [6-10]. Earlier studies comparing autologous SCT and allogeneic SCT have shown that the latter approach does not offer any advantage, owing to transplantation-related mortality (TRM) [2,11,12]. However, reevaluation of this strategy is warranted, considering the recent improvements in supportive care and the use of reduced-toxicity and reduced-intensity conditioning regimens [13-16]. A large retrospective study recently conducted by the Center for International Blood and Marrow Transplant Research (CIBMTR) analyzed allogeneic SCT from matched donors [13], whereas the focus of our present study was on full haplotype-mismatched family donors. Several groups have described relevant alloreactive effects mediated by natural killer (NK) cells in leukemia, and such effects might be extrapolated to patients with NBL as well. Therefore, we present data from 2 prospective trials to evaluate haploidentical (haplo-) SCT with T and B cell-depleted grafts and a melphalan-based conditioning regimen in pediatric recurrent/refractory NBL. Our aim was to show that haplo-SCT in relapsed NBL is feasible with low TRM and low toxicity and can induce long-term survival in some patients.

Table 1
Patient Characteristics and Graft Composition

Characteristic	All Patients (n = 26)	Study Group 1 (Germany) (n = 15)	Study Group 2 (Sweden) (n = 11)
Sex			
Male	17 (65%)	10 (66%)	7 (63%)
Female	9 (35%)	5 (34%)	4 (37%)
Stage at initial diagnosis (ID)			
IIb	1 (4%)	1 (7%)	0
IV	25 (96%)	14 (93%)	11 (100%)
MYCN amplification			
Positive	5 (20%)	1 (7%)	4 (36%)
Negative	21 (80%)	14 (93%)	7 (64%)
Patients with previous SCTs	22 (85%)	15 (100%)	7 (64%)
Autologous	22 (85%)	15 (100%)	7 (64%)
Matched sibling donor	1 (4%)	1 (7%)	0
Number of relapses			
1	16 (61%)	10 (66%)	6 (55%)
≥2	5 (19%)	3 (20%)	2 (19%)
Never in remission	5 (19%)	2 (14%)	3 (27%)
Type of relapse/progression before haplo-SCT			
Local only	1 (4%)	1 (7%)	0
Metastatic	25 (96%)	14 (93%)	11 (100%)
Bone marrow infiltration			
Yes	17 (65%)	13 (87%)	4 (37%)
No	6 (23%)	2 (13%)	4 (37%)
Unknown	3 (12%)		3 (26%)
CNS affection	4 (15%)	0	4 (36%)
Body weight (kg) at SCT, median (range)	21.4 (12-84)	21 (12-64)	24 (15-84)
Status before haplo-SCT			
Complete remission	3 (12%)	1 (7%)	2 (18%)
Partial remission	17 (65%)	12 (80%)	5 (45%)
Progression or non-response	6 (23%)	2 (13%)	4 (36%)
Graft composition, median no. cells/kg BW (range)			
Total nucleated cells	843×10^6 (96-1737)	889×10^6 (97-1737)	544×10^6 (380-914)
Progenitors (CD 34+)	17.20×10^6 (4-48.2)	19.3×10^6 (4.7-28.5)	13×10^6 (9.6-48)
T cells (CD3+)	68.3×10^3 (5-312)	57.8×10^3 (5-138)	100×10^3 (40-312)
NK cells (CD56+)	107.5×10^6 (14-553)	107.5×10^6 (14-553)	not evaluated

Characteristics of all patients and both study subgroups before haploidentical SCT (haplo-SCT) are shown. Graft composition data refer to haplo-SCT. There were no significant differences between the subgroups regarding sex, disease stage at initial diagnosis, *MYCN* amplification, previous SCT, number and type of relapse, and status before haplo-SCT were detectable (Fisher's exact test).

METHODS

Patients

We investigated 26 patients who were enrolled in 2 prospective phase I/II trials. Study 1 was a multicenter trial conducted by Children's University Hospital in Tübingen, Germany, that included participation of the Children's University Hospitals in Tübingen, Munich, Jena, and Greifswald, Germany and Graz, Austria. Study 2 was performed by the Department of Pediatrics, Skåne University Hospital, Lund, Sweden. Most of the transplantations were performed at the centers in Tübingen (11 patients) and Lund (11 patients). The protocol for study 1 was approved by the local Ethics Committees and by Germany's Paul Ehrlich Institute, and the protocol for study 2 was approved by Lund University's Ethical Review Board for Research Involving Humans (DNr 385/2005). Informed consent was obtained from the patients or their legal guardians.

All patients with metastatic relapsed or MYCN-positive local relapsed NBL or primary refractory NBL enrolled between January 2004 and December 2010 were included in this analysis. All patients received multimodal and intensive initial therapy according to national or international NBL protocols (GPOH NB97 or NB2004 in Germany and Austria; HR-NBL-1 SIOPEN in Sweden), including myeloablative high-dose chemotherapy with autologous stem cell rescue for all high-risk patients (n = 22; Table 1).

Relapse therapywas heterogeneous, including various and repeated cycles of chemotherapy (combinations of topotecan, etoposide, cyclophosphamide, ifosfamide, and carboplatin), metaiodobenzylguanidine (MIBG) therapy (n = 20), radiotherapy (n = 12), isotretinoin maintenance therapy (n = 9), surgery, and experimental anti-GD2 antibody therapy (n = 4). MYCN amplification was seen in 5 patients (Table 1).

All donors were parents (10 mothers and 16 fathers) with ≥2 HLA mismatched loci. Donor evaluation involved determining the eligibility for mobilization, crossmatch testing, and assessment of cytomegalovirus (CMV) serostatus.

Stem Cell Mobilization and T and B Cell Depletion

In study 1, donor peripheral blood stem cells were mobilized by administering granulocyte colony-stimulating factor at 10 μg/kg body weight (BW) per day for 5 days and were then harvested by 1 or 2 leukapheresis procedures. Our goal was to obtain 10 to 20 × 106 CD34+ progenitors/kg BW. T and B cells were depleted using CD3- and CD19- coated microbeads and an automated CliniMACS device (Miltenyi Biotec, Gladbach, Germany) according to the manufacturer's instructions. Large-scale tubing sets and the Depletion 2.1 program were used for cell processing.

In study 2, the same protocol and thresholds as in study 1 were used, except that B cell depletion was achieved in vivo by administering anti-CD20 antibody (rituximab) instead of performing CD19 depletion in vitro. In addition, cultivated donor mesenchymal stem cells were administered to all but 2 patients after haplo-SCT to facilitate engraftment [17].

Treatment Protocol

For 24 of the 26 patients, the conditioning regimen consisted of fludarabine (25 to 40 mg/m2/day; total dose 125 to 200 mg/m2), thiotepa (2 × 5 mg/kg BW for 1 day), and melphalan (60 to 70 mg/m2/day for 2 days; total dose 120 to 140 mg/m2). One patient did not receive melphalan (due to toxicity considerations), and in another patient, fludarabine was replaced by clofarabine (50 mg/m2 on days −8 to −5). OKT3 was given as graft rejection prophylaxis (0 to 1 mg/kg/day, maximum dose 5 mg, on days −8 to +14). Methylprednisolone was administered on days −8 to −5 (4 mg/kg) and on days −4 to −1 (2 mg/kg) to avoid the side effects of OKT3. Starting on day 0, hydrocortisone (maximum dose, 2 mg/kg) was used to taper steroids until day +11.Mycophenolate mofetil (MMF; 2 × 600 mg/m2) was given as prophylactic immune suppression (recommended duration, 60 days). High dose MIBG therapy was administered in 13 patients between 15 and 44 days (median, 20 days) before SCT, with an intended dosage of 12 mCi/kg. Exact measurement revealed a mean dose of 11.4 ± 2.8 mCi/kg.

Assessment of Engraftment, Chimerism and GVHD

The day of engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) >500/μL, and platelet recovery was defined as

independence from platelet substitution for at least 7 days with a platelet count ≥20,000/μL.

Chimerism was assessed in peripheral blood by polymerase chain reaction (PCR) analysis of variable number tandem repeat regions or by flow cytometry with monoclonal antibodies against HLA antigens [18,19].

Acute GvHD was graded according to the Glucksberg criteria [20], and chronic GVHD was staged as limited (grade 1) or extensive (grade 2) according to the Seattle criteria [21].

Supportive Care

All patients received prophylactic liposomal amphotericin B or caspofungin, cotrimoxazole, and metronidazole. Immunoglobulins were administered intravenously each week until day +30 and subsequently every 3 weeks until day +100. Recipient-donor pairs who were seronegative for CMV received prophylactic aciclovir until day +180. Patients seropositive for CMV were given pretransplantation ganciclovir prophylaxis and posttransplantation foscarnet prophylaxis, followed by valganciclovir until day +180 or until the CD4 count was >100/μL. Seronegative patients with seropositive donors received only post-transplantation foscarnet prophylaxis, followed by valganciclovir. PCR screening for CMV in blood was performed weekly. In the event of positive findings, foscarnet dosage was increased or the treatment was changed to ganciclovir. Adenovirus (ADV) surveillance consisted of weekly determinations of antigen and PCR screening of stool samples; if the results were positive, PCR analysis of blood was performed. Additional PCR screening for human herpesvirus 6 and Epstein-Barr virus (EBV) was performed during febrile episodes. No prophylactic defibrotide was given.

Donor Lymphocyte Infusion

Single or subsequent donor lymphocyte infusions (DLIs; 2.5 to 5 × 104 CD3+ cells/kg BW) were given to 15 patients to treat residual recipient chimerism or to induce GVT effects. A median of 2 DLIs (range, 1 to 8) were administered per patient. Residual recipient chimerism was treated in patients who demonstrated

increasing autologous signals in the T cell compartment (detected on 2 subsequent flow cytometry-based chimerism analyses) or who exceeded a threshold of >5% autologous T cells. If autologous signals decreased after 3 weeks, no additional DLI was given. If autologous signals persisted or increased, the CD3+ cell dose was doubled the following week. DLI for induction of GVT effects was administered at the treating physician's discretion.

Maintenance Therapy in Long-Term Survivors

Two patients were treated with low-dose IL-2 (1 × 106 U/m2) at the request of their parents. All other patients were in remission without further treatment.

Statistical Analysis

We estimated 95% confidence intervals (CIs) for proportions using the method of Agresti-Coull [22]. Event-free survival (EFS), overall survival (OS), and time to ANC recovery were estimated by the Kaplan-Meier method. We used the log-rank test to compare survival curves between groups. The cumulative incidence of virus reactivation was calculated using the 1 minus Kaplan-Meier method, not adjusted for competing risks. Data analysis was performed using SPSS version 20 (IBM, Armonk, NY) and GraphPad Prism version 5 (GraphPad Software, La Jolla, CA).

Table 2
Engraftment, GVHD, and Side Effects

Parameter	Value	
Engraftment		
Time to ALC > 1×10^9 cells/L (days), median (range)	11	(9-16)
Time to ANC >.5 × 10^9 cells/L (days), median (range)	11	(9-14)
Independence from platelet transfusion (days), median (range)	9	(6-37)
Primary engraftment, n (%)	25	(96)
Rejection, n (%)	1	(4)
Acute GVHD		
Grade, n (%)		
0	4	(15)
1	11	(42)
2	8	(31)
3	3	(12)
4	0	
Organ involvement, n		
Skin only	17	
Skin and gut	4	
Gut only	1	
Liver	0	
Chronic GVHD, n	25	
Grade, n (%)		
None	15	(60)
Limited (skin only)	7	(28)
Extensive (skin and organ involvement)	3	(12)
Organ involvement, n		
Skin only	7	
Gut only	2	
Skin, gut, and liver	1	
Toxicities and infections		
Toxicity (grade 3-4), n (%)		
Gastrointestinal	21	(81)
Stomatitis	21	(81)
Diarrhea	2	(8)
Nausea	13	(50)
Hepatic		
Transaminases/bilirubin	7	(27)
Veno-occlusive disease	0	
Cardiovascular	0	
Neurologic	0	
Pulmonary	1	(4)
Dermal	0	
Renal	1	(4)
Infections, n (%)		
Grade III	2	(8)
Grade IV	0	

Toxicity was graded according to adapted NCI CTC version 3 (Supplementary Table S1). Infection toxicity was determined by grading of fever and treatment-related aspects: grade 3, fever >40°C (<24 hours) and pathogen identified or i.v. antibiotics; grade 4: fever >40°C (>24 hours) and life-threatening hypotension.

RESULTS

Graft Composition

A median of 843 × 106 (range, 96 to 1737 × 106) nucleated cells/kg BWwith 17.2 × 106 (range, 4 to 48 × 106) CD34+ progenitor cells/kg BW and 68 × 103 (range,

5 to 312 × 103) residual CD3+ T cells/kg BW were infused for transplantation. Grafts also included a median of 107 × 106 (range, 14 to 553 × 106) NK cells/kg BW (Table 1).

Engraftment

Primary engraftment was achieved in 25 of the 26 patients (96%). Graft rejection occurred in 1 patient. This patient was rescued with an autologous stem cell backup (Table 2).

Neutrophil engraftment (ANC >500/μL) was reached after a median of 11 days (range, 9 to 17 days). ANC recovery was significantly faster in patients who received granulocyte colony-stimulating factor compared with patients who did not (median, 10 days versus 13 days; $P < .05$). In the 25 evaluable patients, the median time to achieve independence from platelet transfusion was 9 days (range, 6 to 37 days).

GVHD

Fifteen of the 26 patients either did not develop acute GVHD (aGVHD) or developed grade I aGVHD. Eight patients had grade II aGVHD, and 3 patients had grade III aGVHD. No patient had grade IV aGVHD (Table 2). This analysis also included patients who developed GVHD after DLI (n = 15).

Patients were considered evaluable for chronic GVHD (cGVHD) if they engrafted and survived for >100 days. Data on cGVHD were obtained during follow-up (n = 25). Ten patients had cGVHD (7 with grade 1 [28%] and 3 with grade 2 [12%]), but there was no cGVHD-associated mortality. Fisher's exact test revealed no significant differences between the 2 study groups regarding grade II-IV aGVHD or extensive cGVHD. Prophylactic MMF was given to all patients for a median duration of 41 days (range, 19 to 214 days; recommended duration, 60 days) according to decision of the treating physician. MMF was discontinued earlier in cases of increasing mixed chimerism and continued if GVHD occurred.

Toxicity

Toxicity was evaluated according to adapted National Cancer Institute Common Terminology Criteria (NCI CTC), version 3 (Table 2). Twenty-one patients (81%) suffered from grade 3 or 4 gastrointestinal side effects: mucositis in 81% and nausea in 50%. Seven patients (27%) exhibited elevated liver enzymes (serum glutamic oxaloacetic transaminase/glutamic pyruvic transaminase; >3 times the upper limit of normal [ULN]) and/or elevated bilirubin (>5 times the ULN). Transient hypoxia requiring oxygen supplementation occurred in 3 patients. No neurologic, cardiac, renal, or dermatologic grade 3-4 toxicities were observed. No patient developed veno-occlusive disease.

Infections

Of the 9 patients with grade 3 infection, 3 had Staphylococcus spp. as revealed by the positive blood culture; 2 had Enterobacter cloacae; 1 had Klebsiella oxytoca; and 1 had Escherichia coli. This results in 7 patients with positive blood culture and 2 without any bacteria in the blood culture. No grade 4 life-threatening or lethal infections occurred (Table 2).

The cumulative incidence of CMV DNAemia at 1 year was 25% for all patients. No CMV disease occurred. Screening for ADV DNAemia revealed a cumulative incidence of 10% (Figure 1). No EBV reactivation was found.

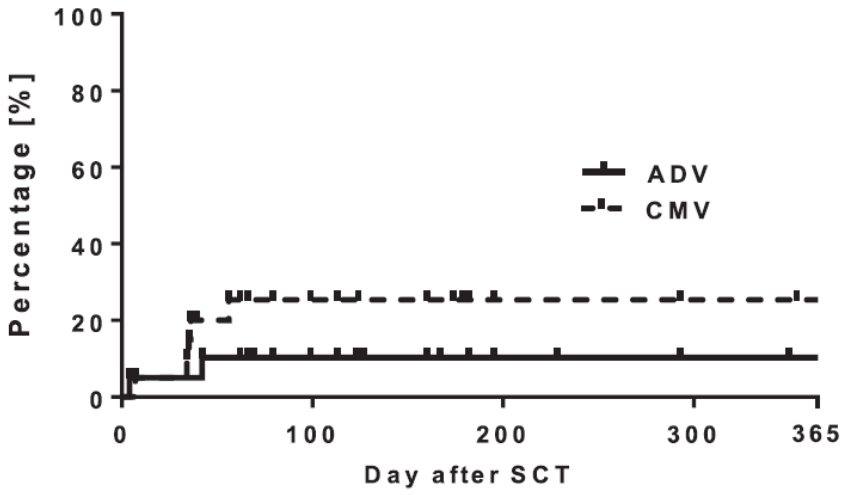

Figure 1. Cumulative incidence of virus reactivation. Patients were screened for ADV-DNA (blood) and for CMV-DNA (blood), and cumulative incidences of positive findings are shown for the entire patient group over a 1-year period.

Survival

As of January 2015, 6 of the 26 patients (23%) were still alive after a median follow-up of 8.1 years (range, 4.91 to 9.28 years). OS and EFS at 5 years were 23% (95% CI, 11% to 42%) and 19% (95% CI, 8% to 38%) respectively, as estimated by the Kaplan-Meier method (Figure 2A and B). Cause of death was progression in 17 patients and relapse in 3 patients. No treatment-related deaths occurred. Our study was not designed to prove equivalence between the 2 study groups; however, Figure 2D does not indicate a clinically meaningful difference in EFS between study groups 1 and 2, respectively.

Disease status before SCT had a statistically significant impact on survival. Patients who achieved a complete remission had a significantly better prognosis than those with residual tumor load (partial remission, Figure 2C; P < .01, logrank test). Moreover, all patients with progressive or nonresponding disease before SCT relapsed within the first year after SCT.

No statistically significant influence on EFS was found for MYCN status (20% versus 19%; P = .87), application of DLI (20% versus 18%; P = .24) and occurrence of aGVHD (14% versus 50%; P = .19).

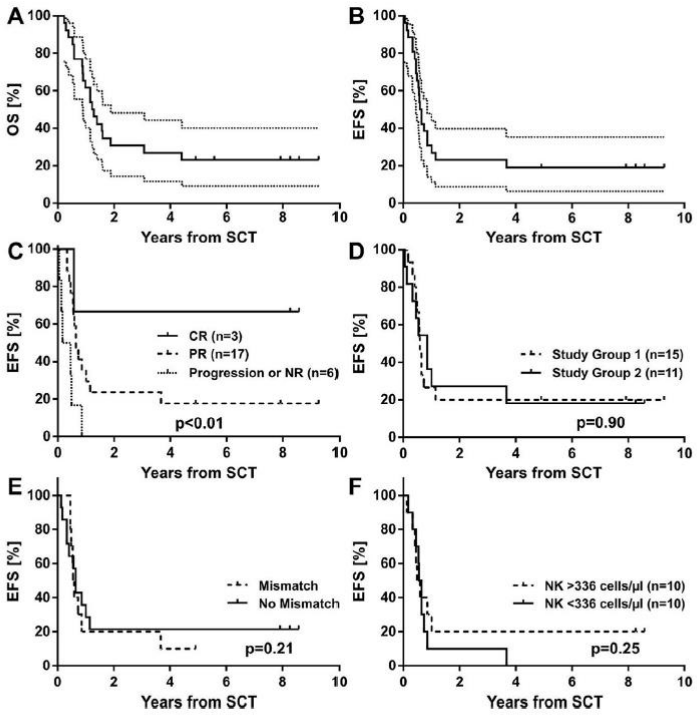

Figure 2. OS and EFS estimated by the Kaplan-Meier method. (A and B) OS (A) and EFS (B) for all 26 patients. The dashed line indicates the 95% CI. (C) EFS related to tumor status before haploidentical SCT. (D) Comparison of EFS in the 2 study groups ($P = .97$). (E) EFS related to occurrence of KIR mismatch. (F) EFS related to the number of NK cells in peripheral blood on day +30 post-transplantation. Two groups are shown: below and above the median NK cell number of 336/µL.

Relapse

Nearly 75% of our patients experienced relapse or progression during the first year after transplantation, as shown in Figure 2. Only 2 out of 20 relapses occurred later than 1 year after transplantation.

Killer Cell Immunoglobulin-Like Receptor Mismatch

Data for killer cell immunoglobulin-like receptor (KIR) mismatch analysis were available for 24 patients. There was no significant difference in the probability of relapse between mismatched and non-mismatched recipient-donor combinations according to the ligand-ligand model [23] (Figure 2E) or between homozygotic and heterozygotic Cw1 allele recipients according to the homozygosity model published by Pfeiffer et al. [24] (data not shown).We also evaluated whether the NK cell reconstitution at day +30 influenced the outcome, and found no difference between patients with NK cell levels below and those with NK cell levels above the median of 336 cells/μL (Figure 2F).

Immune Reconstitution

Recovery of T cells started on day +30 after SCT (mean, 13 cells/μL; range, 0 to 20/μL) and reached 304 cells/μL (range, 8 to 1660 cells/μL) by day +90 Figure 3). A median of 59 days was required to reach a CD3+ cell count >100/μL. Complete recovery of T cell counts occurred by 1 year after SCT. In contrast, the recovery of CD56+ NK cells was rapid; proliferation started during the second week, with a mean of 170 cells/μL on day +14 and 391 cells/μL on day +30. This NK cell elevation persisted for >1 year. A median of only 21 days was required to reach >200 NK cells/μL. The mean number of B cells was 0.4/μL on day +30 and 93/μL on day +120. The median time to reach >200 B cells/μL was 172 days.

Reconstitution of B cells was slower after in vivo depletion (rituximab) than after immunomagnetic ex vivo depletion. On day +90, CD19+ cells numbers reached 35/μL (immunomagnetic) and 0/μL (in vivo). Reconstitution after rituximab started at day +90, ultimately resulting in 84 CD19+ cells/μL (rituximab) versus 100 cells/μL (ex vivo) at day +120.

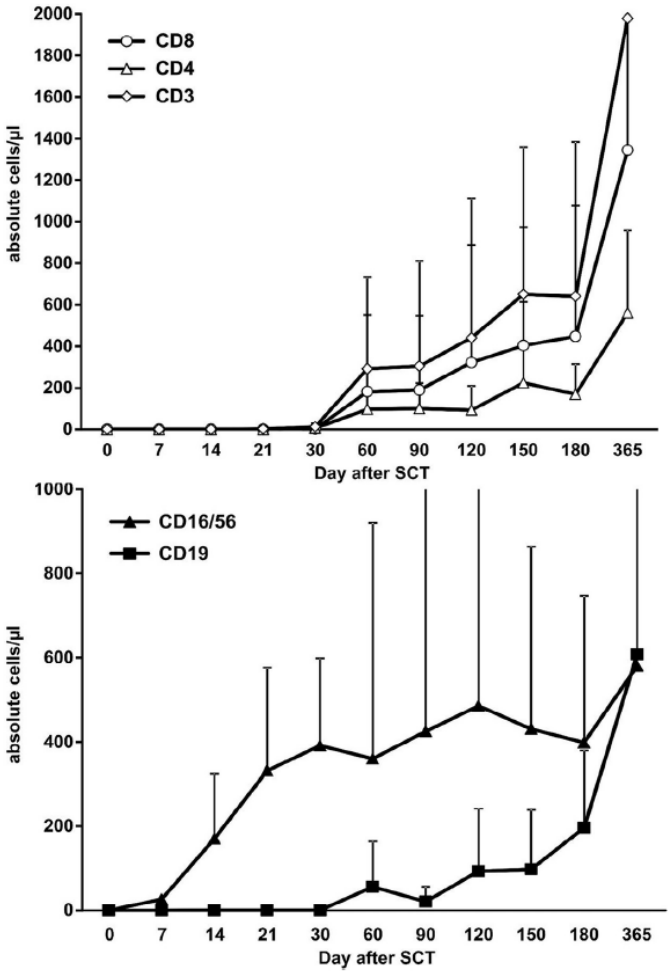

Figure 3. Immune reconstitution. Absolute numbers of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$, and $CD56^+$ T, B, and NK cells after SCT. Data points represent mean ± SD values over a 1-year period.

DISCUSSION

Transplantation of full haplotype-mismatched stem cell grafts has gained wide acceptance in the treatment of leukemias [25-27], and NK cell-mediated alloreactive graft-versus-leukemia reactions have been shown to significantly reduce relapse rates [23]. In our experience, the use of CD3/CD19-depleted grafts from haploidentical donors in combination with reduced-intensity conditioning regimens have been particularly advantageous regarding immune reconstitution and TRM [28,29]. Thus, we considered whether this approach also might be feasible in intensively pretreated patients with refractory/relapsed NBL, and whether NK cell-mediated GVT effects might help improve the prognosis.

In the present analysis, we combined data from 2 similar prospective clinical trials. Both trials investigated the use of T and B cell-depleted haploidentical stem cells after treatment with a melphalan-based myeloablative and reduced toxicity conditioning regimen in comparable study populations. Two differences should be mentioned, however.

First, B cell depletion was achieved by ex vivo graft manipulation in the German study, but by in vivo B cell lysis with rituximab in the Swedish trial, which resulted in a more rapid recovery of CD19+ cells in the early post-transplantation phase in the German patients. Second, mesenchymal stem cells were given on a routine basis only in the Swedish cohort [28].

Nonetheless, from a clinical perspective, the patients and transplantation protocols were comparable in the 2 trials. Thus, we suggest that merging the data is justified to present one of the largest patient cohorts investigated on this topic to date.

Although most patients had previously undergone autologous transplantation, our conditioning regimen was well tolerated without TRM. This compares favorably with a retrospective review of 143 allogeneic SCTs from matched related and unrelated donors conducted by the CIBMTR, which found a TRM of 25% at 1 year [13]. In our patients, rapid recovery of both neutrophils and platelets may have contributed to the moderate toxicity and acceptable infection rate. In contrast, recovery of T cells was delayed compared with SCT from matched sibling donors or with autologous SCT. However, the incidence of viral reactivation was not higher than that reported in another pediatric study in which patients primarily received undepleted grafts from matched donors [30]. The excellent NK cell recovery, which may have mediated a first line defense [31], together with pharmacologic prophylaxis against CMV and intensive surveillance of viral reactivation by PCR with consecutive preemptive therapy against ADV, BK virus, or EBV, may have contributed to these results.

The 42% incidence of grade II-IV aGVHD observed in the present study is higher than that found in patients with acute leukemia in an earlier investigation using an identical conditioning regimen and graft manipulation approach [29], likely due to additional post-transplantation DLIs given to our patients. Because neither DLI

nor occurrence of GVHD was associated with an improved outcome, it is questionable whether T cells can mediate appropriate GVT effects in this context is questionable. Correspondingly, no relationship between the occurrence of GVHD and the outcome of NBL was demonstrated in the CIBMTR review [13] and in a report by Pession et al. [32].

Notwithstanding, there is significant evidence for NK cell-mediated lysis of NBL cells [33], as indicated by a study showing that reduced expression of HLA class I molecules renders most NBL cells susceptible targets for NK cells [34].

Our approach used such effector cells in 2 ways: it allowed infusion of grafts with large numbers of functional active mature donor NK cells immediately after the conditioning regimen, and it led to rapid and strong expansion of donor-derived NK cells within the first 30 days after SCT. However, the presumed GVT effects seemed to be insufficient, because most patients relapsed. Furthermore, we did not observe the significant influence of specific NK alloreactivity based on KIR incompatibilities that Ruggeri et al. [23] previously demonstrated in HLA-mismatched settings.

We hypothesize that these effects might have been masked by the high tumor burden in most of our patients, or that the number of patients was too low to achieve statistical significance. Therefore, in our approach, it is important to induce remission before transplantation. This conclusion concurs with the CIBMTR review, which also showed that disease status before transplantation has a significant impact on outcomes. The 5-year EFS was better in our cohort than in the corresponding group in the CIBMTR review when considering patients who underwent autologous SCT before allogeneic SCT (5-year EFS of 19% and 6%, respectively), but not clearly superior for patients undergoing a second course of high-dose chemotherapy with autologous stem cell support for treatment of relapse (5-year EFS, 15%) [4]. However, antitumor effects might not be sufficient in patients with a large tumor burden. Notwithstanding, haploidentical SCT can serve as a basis for further post-transplantation interventions, because the donor will be available for cellular therapies, and only a short course or no immune suppression is needed.

There is an emerging body of evidence suggesting a role for antibody-dependent cell-mediated cytotoxicity with anti-GD2 antibodies and cytokine stimulation in vitro and in vivo [35-37], and research has already demonstrated a significantly better outcome for patients receiving anti-GD2-based immunotherapy after autologous SCT [38]. These findings strongly suggest that a similar approach can be applied in the allogeneic setting, and preliminary results of a currently ongoing trial indicate that anti-GD2 therapy based on donor-derived NK cells can also increase survival after haplo-SCT [39]. In addition, some patients might not be eligible for a second or third autologous transplantation, because cumulative hematotoxic side effects can make it impossible to subsequently obtain another sufficient stem cell graft.

In summary, for patients with refractory/relapsed NBL, haplo-SCT is a feasible treatment option that can induce long-term remission in some cases. This strategy also may have synergistic potential for post-transplantation therapeutic alternatives based on the donor-derived immune system. Further studies are needed to determine whether such approaches can improve outcomes.

ACKNOWLEDGEMENT

The authors thank Stiftung fuer krebskranke Kinder Tübingen e.V. for continuous support.

Financial disclosure: This work was supported by grants from Deutsche Kinderkrebsstiftung, Deutsche Forschungsgemeinschaft (SFB 685, TP C3), and Reinhold Beitlich Stiftung, awarded to P.L. and R.H.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: J.T., R.H., and P.L. designed the study. J.T., T.F., H.N.L., M.H.A., B.G., C.U., P.L., and R.H. provided patients. M.S. and J.H.D. provided data. T.I., J.T., D.T., and P.L. collected and analyzed data, performed statistical analysis, and wrote the paper. C.P.S., O.T., U.H., H.M.T and M.E. critically reviewed the manuscript. All contributors had access to primary trial data, approved the manuscript, and agree with the presented data

REFERENCES

1. Kushner BH, Kramer K, Cheung NK. Oral etoposide for refractory and relapsed neuroblastoma. J Clin Oncol. 1999;17:3221-3225.
2. Ladenstein R, Pötschger U, Hartman O, et al. 28 years of high-dose therapy and SCT for neuroblastoma in Europe: lessons from more than 4000 procedures. Bone Marrow Transplant. 2008;41(Suppl 2):S118-S127.
3. London WB, Frantz CN, Campbell LA, et al. Phase II randomized comparison of topotecan plus cyclophosphamide versus topotecan alone in children with recurrent or refractory neuroblastoma: a Children's Oncology Group study. J Clin Oncol. 2010;28:3808-3815.
4. Simon T, Berthold F, Borkhardt A, Kremens B, De Carolis B, Hero B. Treatment and outcomes of patients with relapsed, high-risk neuroblastoma: results of German trials. Pediatr Blood Cancer. 2011;56: 578-583.
5. Appelbaum FR. Haematopoietic cell transplantation as immunotherapy. Nature. 2001;411:385-389.
6. Hirayama M, Azuma E, Araki M, Komada Y, Nakagawa A. Evidence of graft-versus-tumor effect in refractory metastatic neuroblastoma. Transplantation. 2006;82:142-144.
7. Inoue M, Nakano T, Yoneda A, et al. Graft-versus-tumor effect in a patient with advanced neuroblastoma who received HLA haplo-identical bone marrow transplantation. Bone Marrow Transplant. 2003;32:103-106.
8. Lang P, Pfeiffer M, Müller I, et al. Haploidentical stem cell transplantation in patients with pediatric solid tumors: preliminary results of a pilot study and analysis of graft versus tumor effects. Klin Padiatr. 2006;218:321-326.
9. Marabelle A, Paillard C, Tchirkov A, et al. Graft-versus-tumour effect in refractory metastatic neuroblastoma. Bone Marrow Transplant. 2007;39:809-810.
10. Toporski J, Garkavij M, Tennvall J, et al. High-dose iodine-131-metaiodobenzylguanidine with haploidentical stem cell transplantation and posttransplant immunotherapy in children with relapsed/refractory neuroblastoma. Biol Blood Marrow Transplant. 2009;15:1077-1085.

11. Ladenstein R, Lasset C, Hartmann O, et al. Comparison of auto versus allografting as consolidation of primary treatments in advanced neuroblastoma over one year of age at diagnosis: report from the European Group for Bone Marrow Transplantation. Bone Marrow Transplant. 1994;14:37-46.
12. Matthay KK, Seeger RC, Reynolds CP, et al. Allogeneic versus autologous purged bone marrow transplantation for neuroblastoma: a report from the Childrens Cancer Group. J Clin Oncol. 1994;12:2382-2389.
13. Hale GA, Arora M, Ahn KW, et al. Allogeneic hematopoietic cell transplantation for neuroblastoma: the CIBMTR experience. Bone Marrow Transplant. 2013;48:1056-1064.
14. Jubert C, Wall DA, Grimley M, Champagne MA, Duval M. Engraftment of unrelated cord blood after reduced-intensity conditioning regimen in children with refractory neuroblastoma: a feasibility trial. Bone Marrow Transplant. 2011;46:232-237.
15. Kanold J, Paillard C, Tchirkov A, et al. Allogeneic or haploidentical HSCT for refractory or relapsed solid tumors in children: toward a neuroblastoma model. Bone Marrow Transplant. 2008;42(Suppl 2):S25-S30.
16. Sung KW. Allogeneic stem cell transplantation for neuroblastoma. Korean J Hematol. 2012;47:3-5.
17. Le Blanc K, Ringdén O. Mesenchymal stem cells: properties and role in clinical bone marrow transplantation. Curr Opin Immunol. 2006;18:586-591.
18. Bader P, Holle W, Klingebiel T, Handgretinger R, Niethammer D, Beck J. Quantitative assessment of mixed hematopoietic chimerism by polymerase chain reaction after allogeneic BMT. Anticancer Res. 1996;16(4A):1759-1763.
19. Schumm M, Feuchtinger T, Pfeiffer M, et al. Flow cytometry with anti HLA-antibodies: a simple but highly sensitive method for monitoring chimerism and minimal residual disease after HLA-mismatched stem cell transplantation. Bone Marrow Transplant. 2007;39:767-773.

20. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graftversus-host disease in human recipients of marrow from HLA-matched sibling donors. Transplantation. 1974;18:295-304.
21. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. Am J Med. 1980;69:204-217.
22. Agresti A, Coull BA. Approximate is better than “exact” for interval estimation of binomial proportions. Am Stat. 1998;52:119-126.
23. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295:2097-2100.
24. Pfeiffer MM, Feuchtinger T, Teltschik HM, et al. Reconstitution of natural killer cell receptors influences natural killer activity and relapse rate after haploidentical transplantation of T- and B-cell depleted grafts in children. Haematologica. 2010;95:1381-1388.
25. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. J Clin Oncol. 2005;23:3447-3454.
26. Ciceri F, Labopin M, Aversa F, et al. A survey of fully haploidentical hematopoietic stem cell transplantation in adults with high-risk acute leukemia: a risk factor analysis of outcomes for patients in remission at transplantation. Blood. 2008;112:3574-3581.
27. Klingebiel T, Cornish J, Labopin M, et al. Results and factors influencing outcome after fully haploidentical hematopoietic stem cell transplantation in children with very high-risk acute lymphoblastic leukemia: impact of center size: an analysis on behalf of the Acute Leukemia and Pediatric DiseaseWorking Parties of the European Blood and Marrow Transplant group. Blood. 2010;115:3437-3446.
28. Chen X, Hale GA, Barfield R, et al. Rapid immune reconstitution after a reduced-intensity conditioning regimen and a CD3-depleted haploidentical stem cell graft for paediatric refractory haematological malignancies. Br J Haematol. 2006;135:524-532.

29. Lang P, Teltschik HM, Feuchtinger T, et al. Transplantation of CD3/CD19 depleted allografts from haploidentical family donors in paediatric leukaemia. Br J Haematol. 2014;165:688-698.
30. Matthes-Martin S, Lion T, Aberle SW, et al. Pre-emptive treatment of CMV DNAemia in paediatric stem cell transplantation: the impact of recipient and donor CMV serostatus on the incidence of CMV disease and CMV-related mortality. Bone Marrow Transplant. 2003;31:803-808.
31. Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. Nat Rev Immunol. 2001;1:41-49.
32. Pession A, Masetti R, Di Leo C, Franzoni M, Prete A. HLA-mismatched hematopoietic stem cell tranplantation for pediatric solid tumors. Pediatr Rep. 2011;3(Suppl 2):e12.
33. Sivori S, Parolini S, Marcenaro E, et al. Involvement of natural cytotoxicity receptors in human natural killer cell-mediated lysis of neuroblastoma and glioblastoma cell lines. J Neuroimmunol. 2000;107:220-225.
34. Bottino C, Dondero A, Bellora F, et al. Natural killer cells and neuroblastoma: tumor recognition, escape mechanisms, and possible novel immunotherapeutic approaches. Front Immunol. 2014;5:56.
35. Koehn TA, Trimble LL, Alderson KL, et al. Increasing the clinical efficacy of NK and antibody-mediated cancer immunotherapy: potential predictors of successful clinical outcome based on observations in high-risk neuroblastoma. Front Pharmacol. 2012;3:91.
36. Ozkaynak MF, Sondel PM, Krailo MD, et al. Phase I study of chimeric human/murine anti-ganglioside G(D2) monoclonal antibody (ch14.18) with granulocyte-macrophage colony-stimulating factor in children with neuroblastoma immediately after hematopoietic stem-cell transplantation: a Children's Cancer Group Study. J Clin Oncol. 2000;18: 4077-4085.
37. Zeng Y, Fest S, Kunert R, et al. Anti-neuroblastoma effect of ch14.18 antibody produced in CHO cells is mediated by NK-cells in mice. Mol Immunol. 2005;42:1311-1319.

38. Yu AL, Gilman AL, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med. 2010;363:1324-1334.
39. Lang P, Illhardt T, Ebinger M, et al. Haploidentical stem cell transplantation and subsequent immunotherapy with antiGD2 antibody for patients with relapsed metastatic neuroblastoma. J Clin Oncol. 2015;33(15 Suppl):10056.

General Discussion and Outlook

The aim of the present work was to investigate the feasibility of SCT in high-risk pediatric solid tumors and present data on the two most common abdominal malignancies in childhood.

In the first study, the possibility of autologous SCT in relapsed or refractory nephroblastoma was investigated. It could be shown that an excellent long-term survival rate without life-threatening side effects could be achieved. However, the number of patients was small, and this form of therapy has already been used successfully by other research groups. Autologous SCT is an established procedure that has been included in several national and international guidelines for the therapy of nephroblastoma. Our study was able to prove the efficacy of this therapy option and thus further underline its use. In addition, our long follow-up period for our patients demonstrated a low risk of recurrence and secondary malignancy as well as a low rate of long-term toxicity. At the time of publication, this was, to our knowledge, the study with the longest mean follow-up period published on this topic.

The second study focused on haploidentical SCT in high-risk neuroblastoma and showed that this therapeutic approach was also feasible in highly pretreated (frequently otherwise palliative) patients with low toxicity and acceptable long-term survival.

Chapters 1 and 2 provide evidence that autologous and haploidentical SCT are feasible approaches to treat pediatric high-risk solid tumors in terms of short- and long-term toxicity, TRM, and prognosis. High-dose chemotherapy in combination with autologous stem cell rescue is an established procedure for a number of solid pediatric tumors and is already found as a standard or salvage therapy suggestion in national and international guidelines for very high-risk (refractory or relapsed) entities [15, 16]. As shown in Chapter 1, a large proportion of patients with nephroblastoma benefit from and remain in complete remission after treatment.

However, there are certain patients who do not respond to the therapy or develop (another) relapse. Therapy options are limited for these individuals and primarily result in palliative care.

Described curative salvage therapies include a second (or subsequent) high-dose chemotherapy with autologous stem cell rescue (so-called *tandem transplantation*) or an allogenic (generally haploidentical) transplantation.

Chapter 2 shows that haploidentical transplantation was able to achieve remission in a large proportion of patients, either temporary or in the long term, without relevant life-threatening side effects. The majority of these patients had received intensive pre-treatment. Most patients (85%) had already undergone high-dose chemotherapy with stem cell rescue at least once. Due to the intensive chemotherapy, another HDC/ASCR is generally associated with significantly more toxicity than a haploidentical transplantation, which can be performed with intensity reduced chemotherapy. Due to the intensive pre-treatment of these patients, serious long-term damage (particularly to the kidneys and liver) or increased TRM is to be expected under repeated intensive chemotherapy. The low rate of relevant GvHD underlines the advantage of haploidentical transplantation over tandem transplantation.

Furthermore, there are increasing signs of positive effects of the newly established immune system in the form of graft-vs-tumor effects. As previously described in the introduction, such effects have already been demonstrated for leukemic diseases, with increasing evidence indicating a transferability of graft-vs-tumor effects to solid tumors [14]. In our cohort, it was not possible to demonstrate such effects reliably. Possible interfering effects might have been low patient numbers, significantly high tumor burden, and inhibition of the immune system by GvHD prophylaxis therapy.

Although these graft-vs-tumor effects cannot be demonstrated with certainty, there is the possibility that the newly established immune system may provide an

ideal starting point for further antibody and cell therapies, as the combination of the new immune system and immunotherapy could generate synergistic effects. In preliminary studies, for example, we were able to show that, in neuroblastomas, after haploidentical SCT, a significant improvement of EFS could be achieved by therapy with anti-GD2 antibodies [17].

Taken together, both approaches, autologous and allogeneic SCT, show certain advantages and disadvantages. The autologous approach (which was analyzed in nephroblastoma patients but has additionally been established in neuroblastoma patients) has short- and long-term sequelae caused by high-dose chemotherapy. Additional risks such as rejection and GvHD are absent. Thus, possible toxicity is exclusively dependent on the type and intensity of chemotherapy. In contrast, immunological anti-tumor effects cannot be expected. Moreover, the successful mobilization of peripheral stem cells is frequently not possible in these heavily pretreated patients.

Future research needs to expand our results on different types of solid tumors, but our studies indicate that haploidentical SCT may be a safe and feasible salvage therapy for other refractory or recurrent solid tumors (for example, additionally, nephroblastoma), particularly when toxicity needs to be prevented or immunotherapy is planned for later.

References

1. Howlader N, N.A., Krapcho M, et al., SEER Cancer Statistics Review (CSR) 1975-2016. Bethesda, Md: National Cancer Institute, 2019.
2. Pasqualini, C., et al., Outcome of patients with stage IV high-risk Wilms tumour treated according to the SIOP2001 protocol: A report of the SIOP Renal Tumour Study Group. Eur J Cancer, 2020. 128: p. 38-46.
3. Smith, V. and J. Foster, High-Risk Neuroblastoma Treatment Review. Children (Basel), 2018. 5(9).
4. Barrett, D., J.D. Fish, and S.A. Grupp, Autologous and allogeneic cellular therapies for high-risk pediatric solid tumors. Pediatr Clin North Am, 2010. 57(1): p. 47-66.
5. Erginel, B., Wilms Tumor and Its Management in a Surgical Aspect, in Wilms Tumor, M.M. van den Heuvel-Eibrink, Editor. 2016: Brisbane (AU).
6. Navalkele, P., et al., Incidence, survival, and prevalence of neuroendocrine tumors versus neuroblastoma in children and young adults: nine standard SEER registries, 1975-2006. Pediatr Blood Cancer, 2011. 56(1): p. 50-7.
7. Maris, J.M., Recent advances in neuroblastoma. N Engl J Med, 2010. 362(23): p. 2202-11.
8. Hale, G.A., Autologous hematopoietic stem cell transplantation for pediatric solid tumors. Expert Rev Anticancer Ther, 2005. 5(5): p. 835-46.
9. Kanold, J., et al., Allogeneic or haploidentical HSCT for refractory or relapsed solid tumors in children: toward a neuroblastoma model. Bone Marrow Transplant, 2008. 42 Suppl 2: p. S25-30.
10. Klingebiel, T., et al., Results and factors influencing outcome after fully haploidentical hematopoietic stem cell transplantation in children with very high-risk acute lymphoblastic leukemia: impact of center size: an analysis on behalf of the Acute Leukemia and Pediatric Disease Working Parties of the European Blood and Marrow Transplant group. Blood, 2010. 115(17): p. 3437-46.
11. Llosa, N.J., et al., Reduced-Intensity Haploidentical Bone Marrow Transplantation with Post-Transplant Cyclophosphamide for Solid Tumors

in Pediatric and Young Adult Patients. Biol Blood Marrow Transplant, 2017. 23(12): p. 2127-2136.

12. Fry, T.J., A. Willasch, and P. Bader, The graft-versus-tumor effect in pediatric malignancy. Pediatr Clin North Am, 2010. 57(1): p. 67-81.
13. Falkenburg, J.H.F. and I. Jedema, Graft versus tumor effects and why people relapse. Hematology Am Soc Hematol Educ Program, 2017. 2017(1): p. 693-698.
14. Lang, P., et al., Haploidentical stem cell transplantation in patients with pediatric solid tumors: preliminary results of a pilot study and analysis of graft versus tumor effects. Klin Padiatr, 2006. 218(6): p. 321-6.
15. van den Heuvel-Eibrink, M.M., et al., Position paper: Rationale for the treatment of Wilms tumour in the UMBRELLA SIOP-RTSG 2016 protocol. Nat Rev Urol, 2017. 14(12): p. 743-752.
16. Sung, K.W., et al., Tandem high-dose chemotherapy and autologous stem cell transplantation in patients with high-risk neuroblastoma: results of SMC NB-2004 study. Bone Marrow Transplant, 2013. 48(1): p. 68-73.
17. Lang, P., Illhardt T., et al., Haploidentical stem cell transplantation and subsequent immunotherapy with antiGD2 antibody for patients with relapsed metastatic neuroblastoma. Journal of Clinical Oncology, 2015. 33(15_suppl): p. 10056-10056.

Erklärung zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde in der pädiatrischen Onkologie der Universitätskinderklinik Tübingen unter Betreuung von Prof. Dr. Peter Lang durchgeführt.
Es handelt sich um eine kumulative Dissertation unter Zusammenführung von 2 klinisch-retrospektiven Publikationen, welche in international anerkannten Journals nach ausführlicher Prüfung in einem „Peer-review“-Verfahren, veröffentlicht wurden.
Erklärungen zum Eigenanteil, und zum Anteil der Co-Autoren, finden sich in den entsprechenden Abschnitten. Ich habe alle statistischen Auswertungen selbstständig durchgeführt und beide Manuskripte mit der Hilfe von Prof. Peter Lang geschrieben.
Die übrigen Teile dieser Dissertation wurden ausschließlich von mir verfasst. Da die Arbeit nicht in meiner Muttersprache geschrieben wurde, habe ich ein kostenpflichtiges Lektorat der Firma *Scribbr B.V. (*Singel 542, 1017AZ Amsterdam) in Anspruch genommen. Inhaltliche Änderungen wurden nicht vorgenommen. Eine Version ohne diese Überarbeitungen liegt dem Promotionsbüro vor.

Toni Illhardt
Tübingen, den 07.11.2020

Publications

Illhardt T, Toporski J, Feuchtinger T, et al. Haploidentical Stem Cell Transplantation for Refractory/Relapsed Neuroblastoma. Biol Blood Marrow Transplant. 2018;24(5):1005-1012. doi:10.1016/j.bbmt.2017.12.805

Illhardt T, Ebinger M, Schwarze CP, et al. Children with relapsed or refractory nephroblastoma: favorable long-term survival after high-dose chemotherapy and autologous stem cell transplantation. Klin Padiatr. 2014;226(6-7):351-356. doi:10.1055/s-0034-1390504

Danksagung

Diese Arbeit wäre nicht ohne die vielen Wegbegleiter möglich gewesen, die mich in vielerlei Hinsicht unterstützt haben.

Mein Dank gilt daher an erster Stelle Prof. Peter Lang, welcher mich als mein Doktorvater in allen wissenschaftlichen Angelegenheiten immer unterstützt hat und beruflich wie auch privat ein großes Vorbild war und ist. Lieber Peter, danke für alles!
Des Weiteren möchte ich allen Kollegen danken, die an den Publikationen mitgearbeitet haben. Ohne meine Co-Autorinnen und -Autoren wären diese Veröffentlichungen nicht möglich gewesen.

Meine Familie war die wichtigste Stütze auf dem langen Weg bis zur Veröffentlichung und das obwohl sie wahrscheinlich selbst nicht mehr daran geglaubt haben, dass ich je fertig werde.
Damit gilt mein weiterer Dank insbesondere meinen Eltern, ohne die ich heute niemals da wäre, wo ich bin. Danke Mama, Danke Papa!
Meine Großmütter haben mit der Aussage „ob ich das noch erlebe?“ den psychologischen Druck immer besonders hochgehalten. Es tut mir leid, dass ich es nicht früher geschafft habe. Seit ich denken kann, seid ihr für mich da gewesen und dafür bin ich euch unendlich dankbar.
Auch beim Rest der Familie möchte ich mich bedanken. Meine Brüder, Onkels und Tanten, Cousins und Cousinen: Ohne eure zynischen Kommentare hätte es nur halb so viel Spaß gemacht.
Meiner Freundin muss ich ebenfalls danken. Dass sie mich in dieser Zeit immer ertragen und unterstützt bedeutet mir viel und war sicher nicht einfach.

Zu guter Letzt gilt ein großer Dank meinen Freunden und Kollegen, die ich hier leider nicht alle persönlich erwähnen kann (Außer Sarah. Danke Sarah!). Ihr habt mir immer mit einem guten Rat zur Seite gestanden und, noch viel wichtiger, für die nötige Ablenkung außerhalb von Klinik und Forschung gesorgt.