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

Vorteilhaftes Langzeitüberleben von Kindern mit rezidivierten Nephroblastomen nach Hochdosis-Chemotherapie und autologem Stammzellrescue

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 (1 200 mg/m²), etoposide (800 mg/m² or 40 mg/kg) and melphalan (180 mg/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.

Zusammenfassung


Methoden: Reinduktionstherapie wurde nach Empfehlung der SIOP-GPOH durchgeführt. Das Konditionierungsregime bestand aus Carboplatin (1 200 mg/m²), Etoposid (800 mg/m² oder 40 mg/kg) und Melphalan (180 mg/m²). Immunomagnetische CD34 positive Selektion wurde bei 5 Patienten durchgeführt.


Schlussfolgerung: Nach unserer Erfahrung ist Hochdosischemotherapie mit autologem Stammzellrescue eine effektive Therapieoption für rezidivierte und refraktäre Nephroblastome mit tolerablen Nebenwirkungen. Jedoch ist eine große randomisierte Studie notwendig um die Effizienz mit hoher Evidenz beweisen zu können.

*Toni Illhardt and Martin Ebinger contributed to this work equally and share first authorship.
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.

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).

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.

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...
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 × 10^6 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 leucocyte count >1 000/µl (ANC) and absolute neutrophile count >500/µl 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). Normalization was reached after one 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).

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.

Table 1: Patient characteristics and initial treatment.

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age at ID (months)</th>
<th>Histologic risk stratification</th>
<th>Initial Stage</th>
<th>Initial Metastases</th>
<th>Treatment prior to HDC/ASCR</th>
<th>Chemotherapy First/Second/Third†</th>
<th>Engraftment</th>
<th>Long term toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>111</td>
<td>III</td>
<td>IV</td>
<td>P, peritoneal seeds (H, Pa), LN</td>
<td>AVD/ICE +</td>
<td>0 (progress)</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>M</td>
<td>59</td>
<td>IV</td>
<td>II</td>
<td>R</td>
<td>AVD/ICE +</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>39</td>
<td>V</td>
<td>III</td>
<td>I, L</td>
<td>AVD/ICE +</td>
<td>2</td>
<td>0 (progress)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>48</td>
<td>I, II</td>
<td>III</td>
<td>L, R</td>
<td>AVD/ICE +</td>
<td>0 (partial remission)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>53</td>
<td>III</td>
<td>II</td>
<td>I, L</td>
<td>AVD/ICE +</td>
<td>+</td>
<td>1</td>
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</tr>
<tr>
<td>6</td>
<td>M</td>
<td>46</td>
<td>I</td>
<td>II–III</td>
<td>I</td>
<td>AVD/ICE +</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>103</td>
<td>III</td>
<td>I</td>
<td>unknown</td>
<td>AVD/ICE +</td>
<td>+</td>
<td>0 (partial remission)</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>11</td>
<td>II</td>
<td>III</td>
<td>II, R</td>
<td>AVD/ICE +</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
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</table>

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, C = Carboplatin, I = Ifosfamide, E = Etoposide, CE = Cyclophosphamide, E = Etoposide, chemotherapy performed according to the national treatment protocol of the German Society for Paediatric Oncology and Haematology.

Table 2: Patient characteristics and initial treatment.

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age at ID (months)</th>
<th>Initial Stage</th>
<th>Initial Metastases</th>
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<tr>
<td>2</td>
<td>M</td>
<td>59</td>
<td>II</td>
<td>R</td>
<td>AVD/ICE +</td>
<td>1</td>
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<tr>
<td>3</td>
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<td>I, L</td>
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<tr>
<td>4</td>
<td>M</td>
<td>48</td>
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<td>I</td>
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<td>0 (partial remission)</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>53</td>
<td>III</td>
<td>I</td>
<td>AVD/ICE +</td>
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</tr>
<tr>
<td>6</td>
<td>M</td>
<td>46</td>
<td>II</td>
<td>II, R</td>
<td>AVD/ICE +</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>103</td>
<td>I</td>
<td>unknown</td>
<td>AVD/ICE +</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>11</td>
<td>II</td>
<td>II, R</td>
<td>AVD/ICE +</td>
<td>+</td>
</tr>
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</table>
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.

Table 2 Relapse, HDC parameters and outcome.

<table>
<thead>
<tr>
<th>ID</th>
<th>Time to Relapse</th>
<th>Site of Relapse</th>
<th>Status before HDC/ASCR</th>
<th>Infused CD34+ cells × 10⁶ per kg BW</th>
<th>CD34-Enrichment</th>
<th>RT after HDC/ASCR</th>
<th>Outcome</th>
<th>Follow-up Interval (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>progression</td>
<td>7.89</td>
<td>—</td>
<td>—</td>
<td>DOD</td>
<td>0.7</td>
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<tr>
<td>2</td>
<td>9</td>
<td>P</td>
<td>CR 2</td>
<td>16.84</td>
<td>+</td>
<td>—</td>
<td>NED</td>
<td>92</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>P</td>
<td>CR 2</td>
<td>7.06</td>
<td>—</td>
<td>+</td>
<td>NED</td>
<td>7.7</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1. local</td>
<td>CR 3</td>
<td>10.2</td>
<td>—</td>
<td>+</td>
<td>NED</td>
<td>124</td>
</tr>
<tr>
<td>5</td>
<td>0*</td>
<td>H</td>
<td>CR 1</td>
<td>18</td>
<td>+</td>
<td>—</td>
<td>NED</td>
<td>127</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
<td>progression</td>
<td>14.41</td>
<td>+</td>
<td>+</td>
<td>NED</td>
<td>102</td>
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<tr>
<td>7</td>
<td>14</td>
<td>P</td>
<td>CR 2</td>
<td>1.91</td>
<td>—</td>
<td>+</td>
<td>NED</td>
<td>101</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>Sp</td>
<td>CR 2</td>
<td>1</td>
<td>+</td>
<td>—</td>
<td>NED</td>
<td>189</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>P</td>
<td>CR 2</td>
<td>15.4</td>
<td>+</td>
<td>—</td>
<td>NED</td>
<td>109</td>
</tr>
</tbody>
</table>

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

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.
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

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, i.e., high-risk histology. Therefore treatment-related mortality might be possible 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 meta-analyses 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 chemotherapeutic 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-enrichment 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.

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