Treatment of high-risk neuroblastoma in children: recent clinical trial results

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Emily Greengard*1, Christine Hill-Kayser2 & Rochelle Bagatell3
1Division of Pediatric Hematology/Oncology, University of Minnesota Amplatz Children’s Hospital, D-557 Mayo Memorial Building, 420 Delaware St, SE, MMC 484, Minneapolis, MN 55455, USA
2Department of Radiation Oncology, The University of Pennsylvania, PA, USA
3Division of Oncology, The Children's Hospital of Philadelphia & The University of Pennsylvania, PA, USA
*Author for correspondence:
Tel.: +1 612 626 2778
Fax: +1 612 626 2815
E-mail: emilyg@umn.edu

Neuroblastoma is the most common extracranial solid tumor in childhood, accounting for more than 7% of malignancies in children younger than 15 years of age and 15% of all pediatric oncology deaths [1]. It is a heterogeneous malignancy with a broad spectrum of clinical behavior. Extensive clinical and basic research over the last four decades has shed light on the biology and treatment of neuroblastoma, but despite remarkable progress, important challenges remain. While some tumors may regress or differentiate spontaneously, others will metastasize widely and result in death despite aggressive multimodality therapy. Chemotherapy and radiotherapy resistance continue to present major therapeutic challenges.

Neuroblastoma risk stratification is based on age at diagnosis, disease stage, tumor histology and tumor biology. The current staging system uses clinical characteristics and imaging defined risk factors to stage the disease as L1, L2, M or MS [2]. Locoregional tumors are designated as either L1 or L2 based on the presence or absence of anatomic characteristics that would make complete surgical excision unsafe or impracticable at the time of diagnosis [3]. Metastatic tumors are defined as stage M, except in cases in which metastases are confined to the skin, liver and/or bone marrow in children younger than 18 months of age. These children are defined as having stage MS disease [2]. Biologic features associated with inferior outcome in patients with neuroblastoma include amplification of the MYCN oncogene, the presence of segmental chromosomal alterations, and diploidy; these tumor characteristics have been incorporated into the current international approach to risk assignment [4]. In general, children who have tumors with MYCN amplification are considered to have high-risk disease. While all children with metastatic disease who were over 12 months of age at the time of diagnosis were previously considered to have high-risk disease even in the absence of MYCN amplification, patients diagnosed between the ages of 12 and 18 months whose tumors have favorable biologic features have been shown to have more favorable outcomes than do older children [5].
Currently, therefore, patients whose tumors are MYCN nonamplified are considered high risk if they are older than 18 months of age at diagnosis with stage M disease. Small subsets of patients <18 months of age may also be considered to have high-risk disease (Table 1) [2].

Despite the aggressive therapy that these children will receive, approximately half will eventually relapse and succumb to their disease. Clinical trials throughout the past decade have improved the outcomes for children with high-risk neuroblastoma; however, it is still evident that novel approaches are needed. The purpose of this review is to summarize the outcomes of recent clinical trials for children with high-risk neuroblastoma.

**Current therapy for high-risk neuroblastoma**

Current therapy for high-risk neuroblastoma is comprised of three main components. The induction phase includes multiagent chemotherapy and surgery; the consolidation phase consists of myeloablative chemotherapy with stem cell rescue followed by external beam radiation and the postconsolidation phase is designed to treat minimal residual disease, and includes both immunotherapy (such as the chimeric 14.18 antibody directed against the disialoganglioside GD2 augmented by granulocyte macrophage stimulating factor and IL-2 in North America, and the anti-GD2 antibody with IL-2 alone in Europe) and a differentiating agent (isotretinoin). This review describes recent clinical trial results for each phase of therapy for patients with high-risk neuroblastoma.

**Recent clinical trial results: induction**

The goal of the induction phase of therapy is to reduce overall disease burden. This is accomplished using intensive, multiagent induction chemotherapy followed by surgical intervention to achieve local control of the disease.

### Table 1. The International Neuroblastoma Risk Group staging system.

<table>
<thead>
<tr>
<th>INRG stage</th>
<th>Age (months)</th>
<th>Histologic category</th>
<th>Grade of tumor differentiation</th>
<th>MYCN</th>
<th>11q aberration</th>
<th>Ploidy</th>
<th>Pretreatment risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1/L2</td>
<td>Any</td>
<td>GN maturing, GNB intermixed</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Very low</td>
</tr>
<tr>
<td>L1</td>
<td>Any</td>
<td>Any, except GN maturing or GNB intermixed</td>
<td>Any</td>
<td>NA</td>
<td>Any</td>
<td>Any</td>
<td>Very low</td>
</tr>
<tr>
<td>L1</td>
<td>Any</td>
<td>Any, except GN maturing or GNB intermixed</td>
<td>Any</td>
<td>AMP</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
</tr>
<tr>
<td>L2 &lt;18</td>
<td>Any, except GN maturing or GNB intermixed</td>
<td>Any</td>
<td>NA</td>
<td>No</td>
<td>Any</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>L2 &lt;18</td>
<td>Any, except GN maturing or GNB intermixed</td>
<td>Any</td>
<td>NA</td>
<td>Yes</td>
<td>Any</td>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>L2 &lt;18</td>
<td>GNB nodular, neuroblastoma</td>
<td>Differentiating</td>
<td>NA</td>
<td>No</td>
<td>Any</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>L2 &lt;18</td>
<td>GNB nodular, neuroblastoma</td>
<td>Differentiating</td>
<td>NA</td>
<td>Yes</td>
<td>Any</td>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>L2 ≥18</td>
<td>GNB nodular, neuroblastoma</td>
<td>Poorly differentiated, undifferentiated</td>
<td>NA</td>
<td>Any</td>
<td>Any</td>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>L2 ≥18</td>
<td>GNB nodular, neuroblastoma</td>
<td>Poorly differentiated, undifferentiated</td>
<td>AMP</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>M &lt;18</td>
<td>Any</td>
<td>Any</td>
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<td>Any</td>
<td>Hyperdiploid</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>M &lt;18</td>
<td>Any</td>
<td>Any</td>
<td>NA</td>
<td>Any</td>
<td>Diploid</td>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>AMP</td>
<td>Any</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>M ≥18</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>MS &lt;18</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>NA</td>
<td>No</td>
<td>Very low</td>
<td></td>
</tr>
<tr>
<td>MS &lt;18</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>NA</td>
<td>Yes</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>MS &lt;18</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>AMP</td>
<td>Any</td>
<td>High</td>
<td></td>
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</table>


Adapted from [4].
primary tumor. In North America, induction chemotherapy regimens being incorporated into current clinical trials are based on a series of studies conducted at Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY, USA). The N6 protocol developed at MSKCC alternated courses of cyclophosphamide, doxorubicin and vincristine with cisplatin and etoposide for a total of seven cycles. In a single institution study of the N6 induction regimen (n = 24), 87% of patients achieved a complete (CR) or very good partial (VGPR) response [6]. A reduction from seven to five cycles of chemotherapy (the N7 protocol) resulted in similar outcomes and less toxicity, with a CR/VGPR rate of 79%, again in a single-center trial [7].

Based on these results, the Children’s Oncology Group (COG) incorporated a modified N6 regimen (six cycles of chemotherapy) into the high-risk neuroblastoma trial A3973. In the cooperative group setting, the end-induction CR + VGPR rate following this therapy was 52%. This was similar to results from a multicenter trial performed by the French Society of Pediatric Oncology, in which 21 out of 47 patients who received this induction regimen achieved CR at metastatic sites [8]. Data from both the COG A3973 and French Society of Pediatric Oncology trials suggest that chemotherapy resistance remains an obstacle. Hematopoietic and mucosal toxicity prevent further intensification of induction chemotherapy, leading investigators to study the addition of newer agents to further improve response rates. The safety and feasibility of the addition of two cycles of topotecan and cyclophosphamide prior to five cycles of N7 chemotherapy were demonstrated in the COG pilot study ANBL02P1 [9]. This induction regimen has recently been studied further in the Phase III COG study ANBL0532; results of this trial are expected in the near future.

In Europe, investigators have studied the concept of rapid administration of maximum tolerated doses of chemotherapy agents in order to induce more rapid cell death and decrease the opportunity for drug resistance. In 2008 Pearson et al. published the results of a trial in which chemotherapy was administered at 10-day intervals, alternating more myelosuppressive regimens (vincristine, carboplatin and etoposide, or vincristine, cyclophosphamide and etoposide) with less myelosuppressive regimens (vincristine and cisplatin) [10]. This rapid regimen (cisplatin, vincristine, carboplatin, etoposide and cyclophosphamide, known as rapid COJEC) was compared with a conventional regimen that utilized the same agents administered every 21 days in a randomized trial, the European Neuroblastoma Group Fifth Study. There was no difference in 3-year event-free survival (EFS) or overall survival (OS) when the two groups were compared; however differences in 5-year EFS were statistically significant (18% in the standard group and 30% in the rapid group; p = 0.02) [10]. In addition, myeloablative therapy was given a median of 55 days earlier in patients assigned to the rapid treatment than those assigned to standard treatment. As expected, infectious complications and time in the hospital were greater with rapid treatment [10].

To reduce the incidence of febrile neutropenia and infectious complications during rapid COJEC induction, a follow-up study, European HR-NBL1/SIOPEN, randomly assigned patients to primary prophylactic versus symptom-triggered GCSF. In this trial, patients on the prophylactic colony stimulating factor arm had significantly fewer febrile neutropenic episodes, days with fever, hospital days and antibiotic days. Rapid COJEC with prophylactic growth factor support is now the standard of care for induction therapy for newly diagnosed children with high-risk neuroblastoma in SIOPEN institutions [11].

- **Recent clinical trial results: consolidation**

  Myeloablative therapy with stem cell rescue was shown to improve EFS in children with high-risk neuroblastoma in the late 1990s and results were confirmed with long-term follow up 10 years later [12,13]. In the Children’s Cancer Group 3891 study, patients were randomized to receive postinduction therapy with either continuation chemotherapy or total body irradiation (TBI) followed by carboplatin, etoposide and melphalan (CEM) and an infusion of stem cells derived from autologous bone marrow. Among patients assigned to autologous transplantation, 3-year EFS (from the time of randomization) was 34%, compared with 22% for patients assigned to continuation chemotherapy [13]. At 5 years, EFS was 30% for patients assigned to the transplant arm and 19% for patients assigned to continuation chemotherapy [14].

  The German NB97 study also evaluated the use of myeloablative consolidation therapy in high-risk neuroblastoma. Patients were randomized to undergo either myeloablative therapy consisting of CEM, or continuation therapy (oral cyclophosphamide) following an intensive induction [15]. The difference in 3-year OS between the two groups did not reach the level of statistical significance in an intention to treat analysis (62% for the transplant group vs 53% for the continuation chemotherapy group; p = 0.09); however, the difference in 3-year EFS was significant (47 vs 31%; p = 0.02). Randomization was stopped due to the improved outcome in the transplant group and excessive toxicity in the continuation chemotherapy group. Importantly, the transplant preparative regimen in this trial included chemotherapy only, indicating that the favorable results of intensified therapy could be achieved without TBI.
Although patients randomized to peripheral blood stem cell transplant (ASCT) who had persistence of metaiodobenzylguanidine (MIBG) avid lesions at the end of induction therapy received therapeutic MIBG, this study nonetheless provided support for the concept of consolidation therapy without external beam TBI.

The results of the European Neuroblastoma Study Group-1 trial confirmed the finding that a non-TBI containing a transplant preparative regimen could be used in children with neuroblastoma [16]. Differences in survival between those who underwent transplant and those who received no additional therapy were not statistically significant for the cohort as a whole, however among children over 1 year of age with international neuroblastoma staging system stage 4 disease, an improvement in 5-year EFS in patients treated with melphalan and autologous stem cell rescue was observed (33 vs 17%; p = 0.01) [16]. Data comparing TBI-containing preparative regimens to chemotherapy-only regimens are limited. However, in recent years there has been increased recognition of late effects related to TBI in survivors, including growth abnormalities, cataracts, thyroid disease and second malignancies [17]. Results of a large (n = 4098) retrospective study indicate that there is no clear improvement in outcome attributable to inclusion of TBI in autologous transplant conditioning for children with neuroblastoma [18] and chemotherapy-only preparative regimens are now used in most cooperative group trials.

The chemotherapy-only preparative regimen most commonly used in North America, CEM, was initially evaluated in a limited institution study (91LA6). The 3-year EFS of 49% observed in patients who received CEM after achieving stable disease or better during induction (n = 71) led to further study of this preparative regimen in the context of the COG trial A3973 [19]. A total of 368 patients completed CEM conditioning for ASCT on A3973. Though the toxic death rate (3%) and the rate of renal failure requiring dialysis (<1%) were low and the 2-year EFS was 48%, CEM was associated with significant toxicity, including severe mucositis in nearly 75% of patients [20].

In Europe, other preparative regimens including busulfan-containing regimens have been studied more extensively. A multivariate analysis of retrospective data generated through the European Bone Marrow Transplant Registry suggested that busulfan-containing regimens were associated with improved outcomes [18]. Based on these data, the International Society of Paediatric Oncology European Neuroblastoma (SIOPEN) HR NBL-1 randomized Phase III study directly compared a busulfan-melphalan (BuMel) preparative regimen with CEM. Although only 598 out of the 1577 patients enrolled on the study underwent randomization, among those who were randomized the 3-year EFS for those assigned to receive BuMel was 49% compared with 33% for those assigned to CEM (p < 0.001). Relapse was less common among those randomized to BuMel rather than CEM, and OS at 3 years was higher among those randomized to the BuMel arm (61 vs 48%; p = 0.004) [21]. The incidence of oral mucositis, gastrointestinal toxicity, ototoxicity, infection and renal toxicity was lower among patients treated with BuMel compared with those treated with CEM. However, clinically relevant sinusoidal obstruction syndrome (SOS) occurred in 18% of patients on the BuMel arm compared with 4% on the CEM arm. Furthermore, outcomes for patients randomized to receive CEM were inferior to those reported in other trials of this preparative regimen [20]. Thus, while BuMel has now become the standard transplant preparative regimen for SIOPEN centers, it is still being evaluated as a component of therapy in other cooperative groups.

Further intensification of therapy through use of sequential autologous transplants has also been studied. The LCME2 trial demonstrated that consecutive cycles of ASCT could be delivered and additional pilots demonstrated the feasibility of this approach [22–30]. The largest trial of tandem transplantation published to date included 97 patients, 82 of whom underwent two consecutive courses of myeloablative therapy (one TBI-containing preparative regimen and one chemotherapy-only preparative regimen) [31]. The 7-year progression-free survival and OS rates of 45 and 53% provided the impetus for a large randomized study of single versus tandem transplant through the COG (ANBL0532). The results of ANBL0532 will also inform current thinking about the role of myeloablative therapy in the context of current-era induction regimens as well as postconsolidation therapy.

Most early studies of stem-cell transplants in children with high-risk neuroblastoma were performed using autologous bone marrow as the stem cell source. Studies of allogeneic transplantation have also been performed, but concerns have been raised about transplant-related mortality (TRM) in the allogeneic setting. A retrospective study has shown that while TRM dropped from 11% prior to 1995 to 4% after this time in patients with neuroblastoma who underwent autologous transplant, the 16% incidence of TRM in neuroblastoma patients undergoing allogeneic transplantation was unchanged over this time period. Furthermore, 5-year progression-free survival was significantly higher in patients who underwent autologous rather than allogeneic transplantation in this cohort [18]. Reduced intensity conditioning has the potential to reduce allogeneic TRM, but further study is needed before allogeneic transplant becomes more widely used in children with neuroblastoma.
While bone marrow was used most widely in early studies of ASCT for neuroblastoma, a transition to the use of peripheral blood stem cells (PBSCs) was made after a series of studies demonstrated the feasibility of harvesting PBSC from small children [32–36]. Harvesting relatively early in induction therapy is recommended so that stem cells are less likely to have been affected by exposure to alkylators and epipodophyllotoxins [37]. Collections are particularly robust when stem cells are harvested after topotecan/cyclophosphamide as initial therapy [9]. In patients who did not undergo harvest with initial therapy, the CXC chemokine receptor type 4 inhibitor plerixafor has been used successfully in small series [38,39]. Concerns regarding contaminating neuroblastoma cells among harvested PBSCs led to studies of ex vivo purging of stem cell products. Positive selection of CD34-expressing cells permits retention of hematopoietic progenitor cells and removal of neuroblastoma cells [40]. However, CD34 selection can cause depletion of T cells and potentially alter immune recovery in patients. Epstein–Barr virus lymphoproliferative disease has been observed in patients who received CD34-selected stem cell products and concerns regarding an increased incidence of serious viral illnesses led to discontinuation of CD34 selection during the COG ANBL00P1 trial of tandem ASCT [30,41]. Negative selection to diminish tumor contamination of stem cell products appeared to be a promising approach based on preclinical data [42]. However, when studied in a large, randomized cooperative group trial, immunomagnetic purging did not improve EFS. A total of 489 children with high-risk neuroblastoma were enrolled on the COG A3973 trial, and 244 patients received stem cell products that had undergone depletion of phagocytes followed by purging using five monoclonal antibodies. The 2-year EFS was 49% in the unpurged group and 47% in the purged group (p = 0.788) [20]. In the absence of improved outcomes in patients receiving purged PBSCs, standard practice no longer includes this additional step in stem cell processing.

Concerns regarding contaminating neuroblastoma cells among harvested PBSCs led to studies of ex vivo purging of stem cell products. Positive selection of CD34-expressing cells permits retention of hematopoietic progenitor cells and removal of neuroblastoma cells [40]. However, CD34 selection can cause depletion of T cells and potentially alter immune recovery in patients. Epstein–Barr virus lymphoproliferative disease has been observed in patients who received CD34-selected stem cell products and concerns regarding an increased incidence of serious viral illnesses led to discontinuation of CD34 selection during the COG ANBL00P1 trial of tandem ASCT [30,41]. Negative selection to diminish tumor contamination of stem cell products appeared to be a promising approach based on preclinical data [42]. However, when studied in a large, randomized cooperative group trial, immunomagnetic purging did not improve EFS. A total of 489 children with high-risk neuroblastoma were enrolled on the COG A3973 trial, and 244 patients received stem cell products that had undergone depletion of phagocytes followed by purging using five monoclonal antibodies. The 2-year EFS was 49% in the unpurged group and 47% in the purged group (p = 0.788) [20]. In the absence of improved outcomes in patients receiving purged PBSCs, standard practice no longer includes this additional step in stem cell processing.

Because neuroblastoma is a radiosensitive tumor and because TBI is no longer widely used during conditioning for ASCT, there is interest in the use of the targeted radionuclide 131I-MIBG as a component of consolidation therapy for patients with high-risk neuroblastoma who have MIBG-avid disease. MIBG is a norepinephrine analog that is preferentially taken up by neuroblastoma cells. The myelosuppression associated with doses of above 12 mCi/kg can be clinically significant; stem cell support is often provided for patients following doses greater than this. Single agent 131I-MIBG at a dose of 18 mCi/kg was associated with a high objective response rate of 37% in a Phase II trial for patients with relapsed or refractory neuroblastoma [43]. This effective but myelosuppressive therapy has been integrated into upfront therapeutic regimens for children with high-risk neuroblastoma in several studies. In total, 44 children were enrolled on a Dutch study of 131I-MIBG as the first intervention in newly diagnosed patients with high-risk disease; 39 received at least two infusions of the radionuclide. The majority of these children (34/39) tolerated an interval of 4 weeks between infusions, and a 66% response rate was observed after the two cycles of therapy [44]. A Phase I study of 131I-MIBG followed by CEM demonstrated that this therapy can be delivered to patients with refractory neuroblastoma in the upfront setting [45]. A single institution retrospective study of 131I-MIBG followed by BuMel demonstrated the feasibility of this regimen in a small cohort of patients with refractory neuroblastoma. Of note, one of the eight patients on this study developed severe SOS resulting in death [46]. Given this observation, as well as the higher rate of SOS in the SIOPEN trial using BuMel conditioning, further evaluation of 131I-MIBG followed by BuMel in a larger cohort of patients is warranted. A COG pilot study using this regimen for newly diagnosed patients (ANBL12P1) is ongoing. If the feasibility of this approach is confirmed, the COG will conduct a randomized trial of 131I-MIBG and BuMel SCT.

External beam radiation as a component of consolidation

Although neuroblastoma is a systemic disease, external beam radiotherapy (EBRT) is a part of modern treatment regimens to address residual (gross or microscopic) disease at the primary tumor site and persistent disease after aggressive systemic treatment. Postoperative local/regional failure has been shown to impact OS, and optimal dose and technique for delivery of EBRT have been evaluated in the past decade [47]. In the CCG3891 trial, patients who had incomplete surgical resections of their primary tumors were nonrandomly assigned to receive EBRT. Patients who were to undergo autologous transplant received 10 Gy delivered to the primary site and subsequently received TBI as part of the transplant preparative regimen, bringing the total radiation dose to the primary tumor site to 22 Gy. Patients randomized to receive continuation chemotherapy were to receive 10 Gy to the primary site alone. EBRT was delivered only to patients who had residual tumor following surgery, and therefore it was not surprising that administration of EBRT did not have a statistically significant impact on local recurrence rates either among the overall population of patients who underwent transplant or those who received continuation chemotherapy. However, among the cohort of patients who received EBRT due to the presence of residual disease at the
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primary site, those who received TBI had a decreased rate of local failure compared with those who received 10 Gy to the primary site alone (22 vs 52%; p = 0.022) [48–50]. Retrospective analysis of the German NB97 experience also supports the role of EBRT to residual disease at the primary tumor site in children with high-risk neuroblastoma. Although numbers of patients were small, the 3-year EFS for children over 1 year of age with international neuroblastoma staging system stage 4 disease who received radiation doses of 36–40 Gy to the site of residual tumor (n = 13) was significantly different from that of patients who did not receive EBRT (n = 23) to residual disease at the primary site (85 vs 25%; p = 0.01) [51]. These data are in keeping with decreased risk of local failure reported by other groups [48–50]. Currently, COG and SIOPEN protocols include a total prescription dose of 21 Gy following gross total resection of the primary tumor. The COG ANBL0532 protocol required a boost of 15 Gy to gross residual disease; results from this trial are currently pending.

Most centers in developed nations use x-ray therapy for delivery of the radiation that is planned using three-dimensional imaging (3D-CRT). In a recent publication from SIOPEN, 99 out of 100 patients treated on a recent study received radiation using 3D-CRT techniques. This technique of delivering x-ray therapy does not allow conformity to protect vital organs. As a result, only 48% of patients on the study were treated according to protocol; the remainder had deviations in delivery to the target due to efforts to protect normal tissues [52]. To improve dosimetry to target volumes and protect normal organs, several groups have investigated the use of intensity-modulated x-ray therapy, intensity modulated arc therapy using x-rays, intraoperative x-ray therapy and proton therapy [53–58]. Each of these modalities has potential to decrease dose to organs at risk, namely liver and kidneys, and excellent local control has been demonstrated with each. However, there are drawbacks to each approach. Intensity modulated x-ray therapy may increase the integral radiation dose by creating a low dose radiation bath. Proton therapy and intraoperative x-ray therapy are relatively new modalities that are available at a limited number of centers. No randomized trials have compared the various approaches to delivery of EBRT to the primary tumor bed, and the design and implementation of such trials is impeded by small patient numbers and differences in access across institutions.

EBRT is also typically employed in high-risk neuroblastoma in an attempt to control sites of disease that appear resistant to induction chemotherapy. Modern COG protocols require the delivery of 21 Gy to all sites of metastatic disease (up to five total) that demonstrate residual abnormal uptake of MIBG on postinduction staging studies. The true benefit of this approach is not well understood, as it has not been studied systematically, but is pursued due to recognition of the radiosensitivity of neuroblastoma cells. Kushner and colleagues investigated the use of 21 Gy delivered to patients with refractory cranial disease and found that most patients (13/19) had a major response to radiation, with control of cranial disease exceeding control of disease elsewhere (52 vs 33%); the same group has demonstrated a technique for brain sparing when large portions of the skull require radiation [59,60]. Although TBI is no longer utilized in neuroblastoma, as outlined above, improved outcomes for patients who received TBI also supports the use of EBRT to target areas of refractory disease [12]. Future directions may focus on optimal balance of use of therapeutic I-131 MIBG versus EBRT for metastatic sites that persist after induction chemotherapy.

Recent clinical trial results: postconsolidation therapy

The goal of postconsolidation therapy is to eradicate minimal residual disease. The first agent to be studied for this purpose was isotretinoin. When neuroblastoma cells are exposed to isotretinoin in vitro, they exhibit decreased proliferation and morphologic differentiation. Growth arrest and differentiation in response to isotretinoin have been observed in neuroblastoma cell lines initiated from tumors at the time of progression after chemotherapy, suggesting that resistance to cytotoxic chemotherapy does not induce resistance to isotretinoin [61–63]. The effect of isotretinoin was evaluated in the multicenter setting in a randomized controlled trial (CCG3891). Isotretinoin was given twice daily for 2 weeks every 28 days for a total of 6 months. Patients randomized to receive isotretinoin had a decreased risk of tumor recurrence regardless of prior treatment with myeloablative or conventional chemotherapy [12,13]. This study established the role of a differentiating agent as a component of therapy in the minimal residual disease setting.

Immune based therapy, predominantly in the form of antibody therapy, has been explored for the treatment of neuroblastoma for over two decades. The target of immune therapy to date has been GD2, a disialoganglioside antigen that is expressed on tumors of neuroectodermal origin, including neuroblastoma and melanoma. These tumors express GD2 with relatively little heterogeneity among cells [64]. In normal tissues, GD2 expression is largely limited to neurons, melanocytes and peripheral pain fibers, making it a reasonable target for antitumor therapy [65].

MSKCC investigators have extensively studied the murine IgG3 monoclonal antibody specific for GD2 known as 3F8. Results of a Phase I trial demonstrated
that 3F8 could be administered safely despite acute toxicities including pain, hypertension and urticaria [66]. No long-side effects of immunotherapy were detected [66]. The Phase I trial was followed by a Phase II study of 3F8 alone in 4 patients with stage 4 neuroblastoma, and more recently by a Phase II trial of 3F8 combined with GM-CSF. In the latter study, complete resolution of bone marrow disease was demonstrated in 12 of 15 patients [67].

The chimeric monoclonal anti-GD2 antibody ch14.18 has been studied extensively in the multicenter setting in both Germany (Society for Pediatric Oncology and Hematology) and North America (COG). Initial single agent Phase I trials of ch14.18 in patients with refractory neuroblastoma and osteosarcoma demonstrated that acute toxicities were similar to those of 3F8, and included pain, tachycardia, hypertension, fever and urticaria [68]. Chimeric 14.18 was studied in Cooperative German Neuroblastoma trials NB90 and NB97, in which patients received induction treatment and radiotherapy as well as maintenance chemotherapy or myeloablative high-dose chemotherapy followed by ch14.18 or no immunotherapy. No significant difference in 3-year EFS was observed for patients who received ch14.18 versus those who did not (46 vs 44%; p = 0.314), though 3-year OS was higher for patients receiving antibody (68 vs 57%; p = 0.018) [69]. Importantly, with longer follow up, the difference in OS for patients receiving ch14.18 versus no antibody therapy remained statistically significant (46 vs 34%; p = 0.019) [70].

GM-CSF was added to ch14.18 therapy in an effort to enhance antibody dependent cytotoxicity in a Children's Cancer Group trial [71], and IL-2 was subsequently added to the immunotherapy regimen [72]. The combination of ch14.18, GM-CSF and IL-2 with isotretinoin in the postconsolidation setting was further studied in a randomized Phase III trial in the COG (ANBL0032). Patients enrolled on this trial were randomized to receive either isotretinoin alone or isotretinoin and immunotherapy. The trial was stopped early as survival rates for patients receiving immunotherapy were found to be significantly higher than those for patients who received isotretinoin alone. The 2-year EFS from the time of postconsolidation randomization was 66% for patients assigned to receive ch14.18 and cytokines versus 46% for patients randomized to isotretinoin (p = 0.01). Differences in 2-year OS were also significant (86 vs 75%; p = 0.02) [73]. Based on these results, immunotherapy including ch14.18 with GM-CSF and IL-2 has become the standard of care for children with high-risk neuroblastoma in North America. In Europe, an ongoing study will determine the role of IL-2 as a component of immunotherapy, as patients are being randomized to receive ch14.18 alone or ch14.18 in combination with IL-2. Acute toxicities associated with GD-2 directed therapy are not inconsequential, and a significant number of patients experience severe neuropathic pain, fever, capillary leak syndrome and hypersensitivity reactions. Studies designed to determine which patients might be at higher risk for severe toxicities are in progress, and investigators are working to identify groups of patients that may benefit the most from this therapy. Furthermore, newer approaches to immunotherapy continue to be studied, including a humanized form of ch14.18 fused to IL-2 and a mutated ch14.18 antibody [74,75].

**Novel therapies: ALK inhibition**

ALK is an orphan receptor tyrosine kinase first identified as part of the t(2;5) chromosomal translocation associated with most anaplastic large cell lymphomas and a subset of T-cell non-Hodgkin’s lymphomas [76]. In addition to its role in anaplastic large cell lymphomas, ALK signaling is activated in other cancers through ALK gene mutations or amplification [76]. In neuroblastoma, mutations in ALK are the major cause of hereditary neuroblastoma but can also be somatically acquired in a larger percentage (8–10%) of sporadic cases [77-80]. In addition, ALK is amplified in approximately 4% of high-risk neuroblastoma tumors [76]. In those cases of neuroblastoma in which an ALK mutation or amplification is present, inhibition of ALK is an attractive therapeutic option.

Crizotinib is an orally bioavailable small-molecule inhibitor of the ALK receptor tyrosine kinase that has been studied in preclinical models of neuroblastoma. Crizotinib has been shown to be highly effective in inhibiting ALK kinase activity, resulting in inhibition of tumor growth [79]. Crizotinib was studied as a single agent in a Phase I clinical trial for children with refractory or relapsed solid tumors or anaplastic large cell lymphoma. The maximum tolerated dose of the drug was found to be 280 mg/m²/day [81]. Dose-limiting toxicities associated with crizotinib included neutropenia and liver enzyme elevation. Among the 11 patients enrolled on the trial whose neuroblastoma tumors harbored an ALK mutation, there was one complete response [82]. A Phase II trial to further investigate the efficacy of this agent in patients whose tumors have been shown to have either ALK mutations or amplification of this gene is nearing completion. A Phase I trial designed to assess the toxicity profile of crizotinib in combination with conventional chemotherapy is ongoing. The results of this study will inform plans to incorporate crizotinib into upfront therapy for the subgroup of patients with high-risk neuroblastoma whose tumors have ALK aberrations.
Future perspective
Over the past decades, significant advances have been made in understanding the biology of neuroblastoma and determining which patients are at increased risk for relapse. The addition of high-dose chemotherapy, ASCT, isotretinoin and immunotherapy to standard regimens for treatment of patients with high-risk neuroblastoma has improved outcomes. Challenges remain, however, as nearly half of all newly diagnosed patients with high-risk disease will still experience a relapse [1].

The integration of targeted therapies, such as ALK inhibition, is a promising approach for the relatively small percentage of patients whose tumors harbor alterations in this gene. Much work has been done to identify additional tractable therapeutic targets for neuroblastoma therapy using next-generation sequencing techniques. However, several large studies identified a relatively small number of recurrent somatic alterations that represent therapeutic targets [82–84]. The challenge for investigators now is to integrate findings from these studies with work focused on epigenetic changes in tumors, evaluations of host factors, and analysis of emerging data from recent clinical trials as treatment regimens for children with high-risk neuroblastoma continue to evolve.

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Executive summary

Background
Clinical trials throughout the past decade have improved the outcomes for children with high-risk neuroblastoma; however, novel approaches are still needed.

Recent clinical trial results: induction
Through the use of dose intensive chemotherapy and surgical resection, the goal of induction therapy is to reduce overall tumor burden.

Recent clinical trial results: consolidation
Consolidation therapy includes autologous stem cell transplant and external beam radiation therapy with future studies evaluating the role of metaiodobenzylguanidine therapy during this phase of treatment.

Recent clinical trial results: postconsolidation therapy
In order to eradicate minimal residual disease, the differentiating agent isotretinoin is used during postconsolidation therapy.

Immunotherapy including the antibody Ch14.18 along with cytokines IL-2 and GM-CSF has significantly improved outcomes for children with high-risk neuroblastoma, although toxicity is not negligible.

Novel therapies
Future studies will likely integrate molecularly targeted therapies into frontline therapy for children whose tumors harbor mutations in genes associated with neuroblastoma oncogenesis.
Because the number of oncogenic drivers identified to date is relatively small, investigators must now not only evaluate potentially tractable molecular targets for neuroblastoma therapy, but must also evaluate epigenetic changes in tumors, assess the role of host factors in therapy and analyze emerging data from recent clinical trials of existing agents in order to make further improvements in outcomes for children with high-risk neuroblastoma.

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