Advances in the Diagnosis and Treatment of Neuroblastoma

JOANNA L. WEINSTEIN,a,b HOWARD M. KATZENSTEIN,c,d,e SUSAN L. COHNa,b

aDepartment of Pediatrics and bRobert H. Lurie Comprehensive Cancer Center, Northwestern University, The Feinberg School of Medicine, Chicago, Illinois, USA; cAFLAC Cancer Center, dChildren’s Healthcare of Atlanta, and eEmory University, Atlanta, Georgia, USA

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ABSTRACT

Neuroblastoma, a childhood neoplasm arising from neural crest cells, is characterized by a diversity of clinical behavior ranging from spontaneous remission to rapid tumor progression and death. To a large extent, outcome can be predicted by the stage of disease and the age at diagnosis. However, the molecular events responsible for the variability in response to treatment and the rate of tumor growth remain largely unknown. Over the past decade, transformation-linked genetic changes have contributed to the understanding of tumor predisposition, metastasis, treatment responsiveness, and prognosis. The Children’s Oncology Group recently developed a Neuroblastoma Risk Stratification System that is currently in use for treatment stratification purposes, based on clinical and biologic factors that are strongly predictive of outcome. This review discusses the current risk-based treatment approaches for children with neuroblastoma and recent advances in biologic therapy.

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INTRODUCTION

Neuroblastoma (NB), the most common extracranial solid tumor of childhood, is remarkable for its broad spectrum of clinical behavior [1, 2]. Some NB tumors undergo spontaneous regression or differentiate into benign ganglioneuromas [3-5]. Most children with stages 1 and 2 disease [6] can be cured with surgery alone [7, 8]. In addition, most infants with disseminated disease have favorable outcomes following treatment with chemotherapy and surgery [9, 10]. In contrast, the majority of children older than 1 year of age with advanced-stage NB die from progressive disease despite intensive multimodality therapy.
This clinical diversity correlates closely with numerous clinical and biological factors, including tumor stage, patient age, tumor histology, and genetic abnormalities [12-15]. However, the molecular basis underlying the variability in tumor growth, clinical behavior, and responsiveness to therapy remains largely unknown.

Because outcome is significantly better for patients with localized disease and younger age, many investigators speculated that screening infants for NB would lead to reduced mortality. Pioneering studies performed in Japan in the 1980s demonstrated that NB could be detected by screening for urinary catecholamines at 6 months of age and suggested that preclinical detection led to improved survival [16-18]. However, population-based approaches for screening were not used in these studies and no concurrent control groups were evaluated. Subsequent trials demonstrated that the incidence of diagnosis of NB was increased in Japan and that virtually all tumors detected by screening had favorable biologic features [19, 20]. These observations suggest that many of the tumors detected by screening were likely to undergo spontaneous regression and would never have been diagnosed clinically. To directly answer the question of whether routine screening for NB would result in lower mortality, two prospective population-based, controlled trials were recently conducted in Germany and North America [21, 22]. The studies demonstrated that screening infants for NB at 3 weeks, 6 months, or 1 year did not reduce mortality due to this disease. Furthermore, similar to the previous reports from Japan, almost all tumors detected by screening had favorable biologic features. Thus, there appears to be no role for screening infants for NB.

There are approximately 600 new cases of NB in the U.S. each year, with a prevalence of approximately one case per 7,000 births [23]. This tumor is derived from neural crest cells, and it most commonly arises in the adrenal medulla or paraspinal sympathetic ganglia. The etiology of NB remains obscure. To date, no environmental influences or parental exposures that significantly impact on disease occurrences have been identified [24]. NB usually occurs sporadically; however, in 1%-2% of cases there is a family history [25, 26]. Interestingly, considerable biological and clinical heterogeneity is also observed in the familial cases [27]. While the occurrence of familial NB suggests the presence of a unifying underlying genetic abnormality, studies to date have failed to identify a specific tumor suppressor gene responsible for NB tumorigenesis [15, 27].

CLINICAL PRESENTATION

Presenting signs and symptoms of children with NB reflect both the location of the primary tumor and the extent of disease. Patients with localized disease are often asymptomatic, while children with metastatic disease typically appear ill at presentation with systemic symptoms, including fever and bone pain secondary to tumor dissemination. Metastatic disease to the orbit may manifest as orbital ecchymoses and is commonly mistaken for child abuse. Patients with paraspinal tumors may present with signs of spinal cord compression, while Horner’s syndrome is sometimes observed in individuals with cervical or apical thoracic masses (Fig. 1). Several paraneoplastic syndromes may also be seen at presentation, including opsoclonus, myoclonus, and ataxia. Rarely, tumor secretion of vasoactive intestinal peptide can result in profuse diarrhea.

Figure 1. Computed tomography scan of an apical paraspinal NB in a child who presented with Horner’s Syndrome.
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clinical prognostic factor. For all stages of disease beyond localized tumors, infants less than 1 year of age have significantly better disease-free survival rates than older children with equivalent stages of disease [30, 31]. Additional studies suggest that 1- to 2-year-old children with disseminated disease have a better outcome than children over 2 years of age [32].

Histology

In 1984, Shimada and colleagues devised a classification schema that relates the histopathologic features of the tumor to clinical behavior [33]. Tumors are classified as favorable or unfavorable depending upon the degree of neuroblast differentiation, Schwannian stroma content, mitosis-karyorrhexis index, and age at diagnosis. The International Neuroblastoma Pathology Classification system, a modification of the Shimada system, was established in 1999, and the prognostic significance of this system has been confirmed [14]. Although it remains unknown why unfavorable histology tumors are more clinically aggressive, amplification of the MYCN oncogene is strongly associated with unfavorable histology [34, 35].

Table 1. International neuroblastoma staging system

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached to and removed with the primary tumor may be positive).</td>
</tr>
<tr>
<td>2A</td>
<td>Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically.</td>
</tr>
<tr>
<td>2B</td>
<td>Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically.</td>
</tr>
<tr>
<td>3</td>
<td>Unresectable unilateral tumor infiltrating across the midline,* with or without regional lymph node involvement; or localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement.</td>
</tr>
<tr>
<td>4</td>
<td>Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined by stage 4S).</td>
</tr>
<tr>
<td>4S</td>
<td>Localized primary tumor (as defined for stage 1, 2A, or 2B) with dissemination limited to skin, liver, and/or bone marrow*. Limited to infants &lt;1 year of age.</td>
</tr>
</tbody>
</table>

* The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.

+ Marrow involvement in stage 4S should be minimal, i.e., <10% of total nucleated red blood cells identified as malignant on bone marrow biopsy or on marrow aspirate. More extensive marrow involvement would be considered to be stage 4. The mIBG scan (if performed) should be negative in the marrow.

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Figure 2. 131I-mIBG scan of a patient with stage 4 NB showing an adrenal primary tumor and bone lesions in the femur and spine.
A role for MYCN in NB pathogenesis is further supported by studies demonstrating NB tumor development in transgenic mice with targeted expression of MYCN [42]. Although MYCN amplification clearly identifies a subset of NBs with highly malignant behavior, the clinical significance of greater MYCN expression in children with NB remains controversial [43-46]. The reason for the discordant results may, in part, be due to disparities in patient populations, as the proportions of infants <1 year of age, patients with advanced-stage disease, and children with MYCN-amplified tumors differ in the various series. Recently, high levels of MYCN expression were found not to be predictive of a worse outcome in a retrospective analysis of patients with advanced-stage disease and normal MYCN copy number [47]. Thus, the precise role, if any, that MYCN plays in nonamplified tumors remains unknown.

### Tumor Cell Ploidy

A number of studies have shown that cellular DNA content is predictive of outcome in patients with NB, particularly in infants less than 1 year of age [32, 48-50]. Hyperdiploidy, mostly in near-triploid constitution, is mainly observed in low-stage tumors of infants and is associated with excellent long-term survival. On the other hand, diploidy in this age group is often associated with early treatment failure [35, 48, 51]. While a correlation exists between diploidy and MYCN amplification, each factor has been validated as an independent prognostic variable [32, 35, 50].

### Chromosome Abnormalities

Both gains and losses of genetic material are commonly detected in NB cell lines and primary tumors. The most common genetic abnormality in primary NB is gain of 17q genetic material. This abnormality is strongly associated with high-risk features and adverse outcome [15, 52]. Consistent areas of chromosomal loss of heterozygosity (LOH) include chromosome band 1p36, 11q23, and 14q23-qter [15]. A strong correlation exists between the allelic loss of 1p and high-risk NB features, including older age, metastatic disease, MYCN amplification, and unfavorable outcome [53-55]. Two large independent studies have shown that, while deletion of 1p was associated with unfavorable outcome in a univariate analysis, this factor was not prognostic after adjusting for MYCN copy number [55, 56]. In contrast, Caron and colleagues reported that loss of 1p was predictive of unfavorable outcome, independent of MYCN amplification [54]. Recently, a large Children’s Cancer Group (CCG) study showed that 1p deletion independently predicted for lower event-free survival but not overall survival [57].

In contrast to 1p LOH, 11q LOH and 14q LOH are inversely correlated with MYCN amplification [58-60]. A univariate analysis revealed no survival disadvantage for patients whose tumors had 11q genetic loss. However, within the cohort of patients with normal MYCN copy number tumors, 11q LOH was associated with a significantly lower overall survival probability. The clinical relevance of 14q LOH is not clear.

### Table 2. Prognostic factors in neuroblastoma

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>Favorable</th>
<th>Unfavorable</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>1, 2, 4S</td>
<td>3, 4</td>
<td>[30, 31]</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;365 days</td>
<td>&gt;365 days</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td>Low</td>
<td>High</td>
<td>[165, 166]</td>
</tr>
<tr>
<td>LDH</td>
<td>Low</td>
<td>High</td>
<td>[167]</td>
</tr>
<tr>
<td>NSE</td>
<td>Low</td>
<td>High</td>
<td>[168]</td>
</tr>
<tr>
<td>Histology</td>
<td>Favorable</td>
<td>Unfavorable</td>
<td>[33]</td>
</tr>
<tr>
<td><strong>Biologic Factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYCN oncogene</td>
<td>Normal Copy</td>
<td>Amplified</td>
<td>[36, 37]</td>
</tr>
<tr>
<td>DNA index</td>
<td>&gt;1.0 (hyperdiploid)</td>
<td>1.0 (diploid)</td>
<td>[48, 32]</td>
</tr>
<tr>
<td>Chromosome 1p</td>
<td>Normal</td>
<td>Deletion</td>
<td>[54, 57]</td>
</tr>
<tr>
<td>Chromosome 17q</td>
<td>Normal</td>
<td>Gain</td>
<td>[52]</td>
</tr>
<tr>
<td>TrkA expression</td>
<td>High</td>
<td>Low</td>
<td>[65, 15, 66]</td>
</tr>
<tr>
<td>TrkC expression</td>
<td>High</td>
<td>Low</td>
<td>[67, 68]</td>
</tr>
<tr>
<td>TrkB expression</td>
<td></td>
<td>High/FL</td>
<td>[69]</td>
</tr>
<tr>
<td>CD44 expression</td>
<td>High</td>
<td>Low</td>
<td>[169-171]</td>
</tr>
<tr>
<td>MRP expression</td>
<td>Low</td>
<td>High</td>
<td>[172]</td>
</tr>
<tr>
<td>Vascularity</td>
<td>Low</td>
<td>High</td>
<td>[148, 149, 173, 174]</td>
</tr>
</tbody>
</table>

Abbreviations: LDH = lactate dehydrogenase; NSE = neuron-specific enolase; FL = full-length transcript; MRP = multidrug-related protein.
Neurotrophins and Neurotrophin Receptors

The Trk family of tyrosine kinases are critical mediators of neurotrophin signaling and play an essential role in normal neuronal development [61]. Differential expression of these neurotrophin receptors is highly associated with the variable biologic and clinical characteristics of NB [62]. \textit{trkA} expression is inversely related to disease stage and MYCN amplification status [63], and accordingly, high \textit{trkA} expression is associated with favorable prognosis [64-66]. Similar to \textit{trkA}, \textit{trkC} is also highly expressed in biologically favorable NBs [62, 67, 68]. Conversely, \textit{trkB} is expressed primarily in advanced-stage tumors that are MYCN amplified [69], whereas it is expressed at low levels or in a truncated form in biologically favorable tumors [62, 67, 68].

**RISK GROUP STRATIFICATION SYSTEM**

The Children’s Oncology Group (COG) Neuroblastoma Risk Stratification System is based on the International Risk Grouping System [70], and it is used for treatment stratification purposes. Patients are assigned into low-, intermediate-, and high-risk categories (and accordingly, phase III treatment protocols) based upon an analysis of age at diagnosis, INSS stage, histopathology, MYCN amplification status, and DNA index (Table 3).

**RISK-BASED TREATMENT**

**Low-Risk Disease**

Low-risk NB patients require minimal therapy [71-73]. Previous Pediatric Oncology Group (POG) and CCG studies have shown that treatment with surgery alone resulted in survival (S) rates of >95% for patients with stage 1 disease (Fig. 3) [7, 8]. The management of the infrequent patient with stage 1 or 2 disease with MYCN amplification remains controversial [8, 74, 75]. Although patients with MYCN-amplified stage 1 tumors have significantly worse event-free survival (EFS) and S rates, a subset may achieve long-term remission following surgery alone [7, 74]. These rare cases require continued prospective evaluation to clarify optimal management.

A high rate of spontaneous regression is seen in infants with stage 4S NB, and high survival rates have been reported in 4S infants whose tumors lack MYCN amplification [76, 77]. Interestingly, \textit{Tonini} and colleagues reported that, in the Italian experience, favorable outcome was also seen in infants with MYCN-amplified stage 4S NB [78]. Newborns with small adrenal masses constitute a particularly favorable cohort of patients [79-81]. Recently, \textit{Yamamoto} and colleagues defined criteria for observing NB tumors detected by screening, and to date, all tumors have decreased in size or resolved spontaneously [82]. Others have reported spontaneous regression of adrenal NBs detected antenatally by ultrasound [83]. These observations suggest that newborns with small or cystic localized NBs can be safely observed with a low risk of progression to advanced-stage disease. To prospectively test this hypothesis, the COG recently developed a clinical trial in which infants with small adrenal primary NBs will be observed rather than undergo major surgery.

Excellent outcome is also seen in patients with stages 2A and 2B disease. Four-year EFS and S rates of 81% ± 4% and

<table>
<thead>
<tr>
<th>INSS Stage</th>
<th>Age</th>
<th>MYCN status</th>
<th>Shimada histology</th>
<th>DNA index</th>
<th>Risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-21 years</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Low</td>
</tr>
<tr>
<td>2A/B</td>
<td>&lt;365 days</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>365 days-21 years</td>
<td>Normal</td>
<td>Any</td>
<td>-</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>365 days-21 years</td>
<td>Amplified</td>
<td>Favorable</td>
<td>-</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>365 days-21 years</td>
<td>Amplified</td>
<td>Unfavorable</td>
<td>-</td>
<td>High</td>
</tr>
<tr>
<td>3</td>
<td>&lt;365 days</td>
<td>Normal</td>
<td>Any</td>
<td>Any</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>&lt;365 days</td>
<td>Amplified</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>365 days-21 years</td>
<td>Normal</td>
<td>Favorable</td>
<td>-</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>365 days-21 years</td>
<td>Normal</td>
<td>Unfavorable</td>
<td>-</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>365 days-21 years</td>
<td>Amplified</td>
<td>Any</td>
<td>-</td>
<td>High</td>
</tr>
<tr>
<td>4</td>
<td>&lt;365 days</td>
<td>Normal</td>
<td>Any</td>
<td>Any</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>&lt;365 days</td>
<td>Amplified</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>365 days-21 years</td>
<td>Any</td>
<td>Any</td>
<td>-</td>
<td>High</td>
</tr>
<tr>
<td>4S</td>
<td>&lt;365 days</td>
<td>Normal</td>
<td>Favorable</td>
<td>&gt;1</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>&lt;365 days</td>
<td>Normal</td>
<td>Any</td>
<td>=1</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>&lt;365 days</td>
<td>Normal</td>
<td>Unfavorable</td>
<td>Any</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>&lt;365 days</td>
<td>Amplified</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
</tr>
</tbody>
</table>

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98% ± 1.5%, respectively, were reported in previous CCG studies following treatment with surgery alone [8]. In POG studies, localized disease that was not completely resected (analogous to INSS stage 2A) was treated postoperatively with chemotherapy. The estimated 3-year S rate for patients with hyperdiploid tumors that lacked MYCN amplification was 96% [9]. Similarly, excellent outcomes for patients with localized NBs (defined as INSS stages 1, 2, and 3) were seen in the French NBL 90 Study [75]. These findings support the reduction-in-therapy approach that is being tested in the current COG low-risk study. The overall objective of the COG low-risk study is to preserve the excellent survival rate for patients with low-risk NB by using surgery as the primary treatment approach, thereby minimizing the risks of acute and long-term chemotherapy-related morbidity for the majority of these patients.

Intermediate-Risk Disease

In previous POG studies, treatment for infants with regional and metastatic disease was stratified by MYCN amplification and tumor cell ploidy. Infants with hyperdiploid tumors were treated with cyclophosphamide and doxorubicin, whereas infants with diploid tumors received cisplatin and teniposide after an initial course of cyclophosphamide plus doxorubicin [9]. The most recent analysis of patients enrolled in that study demonstrates estimated 11-year S rates of 94% ± 5% and 52% ± 16% for patients with hyperdiploid and diploid tumors, respectively (personal communication; Wendy London, Ph.D., COG Data Statistical Center, University of Florida, Gainesville, FL). An S rate of 71% ± 7% was reported in a prospective CCG trial in which infants with regional and metastatic disease were treated with a four-drug chemotherapy regimen, surgery, and local radiation to residual disease [10]. Infants with tumors that lacked MYCN amplification had a 93% ± 4% 3-year EFS rate, whereas those with amplified MYCN had a 10% ± 7% 3-year EFS rate ($p < 0.0001$). These results emphasize the clinical significance of NB tumor biology.

Excellent S rates have been reported in patients older than 1 year with favorable biology regional tumors following treatment with surgery and chemotherapy [84, 85]. However, the use of adjuvant therapy for patients with regional disease has been challenged in a single-institution study in which 88% of patients with INSS stages 2B and 3 tumors that lacked MYCN amplification survived without disease progression following surgery alone [86]. These observations suggest that, for the majority of patients with biologically favorable regional tumors, chemotherapy may be safely reduced or eliminated. In an effort to avoid associated acute and long-term complications while maintaining high cure rates, adjuvant chemotherapy and radiotherapy have been reduced in the current COG Intermediate-Risk Study. Intermediate-risk patients with favorable biology tumors are treated with a short course of chemotherapy (four cycles), while intermediate-risk patients with unfavorable biology receive a longer course of chemotherapy (eight cycles).

High-Risk Disease

Survival for high-risk children has improved modestly during the past 20 years, although cure rates remain low [11, 87-89]. This improvement is thought to be due to intensification of induction chemotherapy, megatherapy consolidation, and improved supportive care [90]. Dose-intensity has been shown to correlate strongly with both

![Figure 3. Kaplan-Meier S and EFS curves and life tables for patients with INSS stage 1 NB. Reprinted with permission [7].](image)
response and progression-free survival, and response rates from 70%-80% have been seen with intensive multiagent induction regimens [11, 91]. Furthermore, several single-armed studies have suggested that intensification of consolidation therapy with autologous stem cell transplantation following myeloablative doses of chemotherapy with or without total body irradiation also contributes to improved overall survival [88, 89, 92, 93]. A report from the European Group for Blood and Marrow Transplant of 1,070 myeloablative procedures followed by stem cell rescue performed during the past 17 years found a 2-year posttransplant survival rate of 49% and a 5-year survival rate of 33% [94]. Recently, the superiority of myeloablative therapy and autologous bone marrow transplant over conventional dose chemotherapy was definitively demonstrated in a randomized study conducted by the CCG [11]. In that study, the 3-year EFS rate was significantly better for patients randomized to the transplant arm than for patients randomized to continuous chemotherapy (34% ± 4% versus 22% ± 4%, respectively, $p = 0.034$) (Fig. 4).

In an effort to further dose intensify consolidation therapy, some investigators have treated patients with tandem cycles of high-dose therapy in conjunction with stem cell rescue. Grupp and colleagues conducted a single-arm trial of peripheral blood stem cell (PBSC)-supported tandem transplantation for high-risk NB patients, demonstrating that tandem transplant was feasible in this patient cohort and that toxicity was acceptable [95]. The estimated 3-year EFS rate from the date of diagnosis was promising at 58% (90% confidence interval, 40%-72%). Another pilot study conducted at Children’s Memorial Hospital in Chicago utilized triple-tandem cycles of high-dose therapy with PBSC rescue [96]. With a median of 32 months follow-up, the estimated 3-year EFS rate from the time of diagnosis in that study was 57% ± 11%.

Unfortunately, despite intensive multimodality treatment, more than 50% of children with high-risk disease will relapse due to drug-resistant residual disease [11, 97]. Eradication of refractory microscopic disease remains one of the most significant challenges in the treatment of high-risk NB. In an effort to treat chemotherapy-resistant tumor cells, the differentiation agent 13-cis-retinoic acid (RA) has been administered to high-risk patients following completion of consolidation therapy. A recently completed CCG study demonstrated that administration of this differentiation agent in the setting of minimal residual disease was clinically effective and resulted in a greater 3-year EFS rate (Fig. 5) [11]. Preliminary data suggest that other biologic agents may also be clinically effective in the setting of minimal residual disease (see below).

**TREATMENT OF SPINAL CORD COMPRESSION**

Approximately 7%-15% of NB patients present with paraspinal tumors that extend through vertebral foramina either with or without associated spinal cord compression [98-101]. Prompt resolution of spinal cord foramina may prevent the development of permanent neurologic impairment in these children. Current therapeutic strategies to relieve spinal cord compression include surgical resection either with or without laminectomy, chemotherapy, and radiation therapy [99, 102-105]. The optimal treatment approach for cord decompression, however, remains unknown. In a retrospective review of the POG experience, similar rates of neurologic recovery were observed in symptomatic patients following treatment with chemotherapy or laminectomy, although more orthopedic sequelae were observed in the children treated with...
laminectomy [106]. Plantaz and colleagues similarly reported that chemotherapy effectively relieved neurologic symptoms from cord compression due to NB [107]. Taken together, these results support a primary medical approach for the initial treatment of children with intraspinal NB tumors, with laminectomy reserved for those that fail chemotherapy.

**TREATMENT OF OPSOCLONUS/MYOCLONUS SYNDROME**

The opsoclonus/myoclonus syndrome (OMS) that occurs coincident with NB is believed to be immune mediated. Although approximately 60% of patients respond to adrenocorticotropic hormone or corticosteroid therapy, most patients have recurrences of their neurologic symptoms and develop developmental delays or mental retardation [108, 109]. Several retrospective studies suggest that the administration of chemotherapy may improve the long-term neurologic outcome of this group of patients [110, 111]. There have also been several case reports indicating good responses to treatment with i.v. gammaglobulin [112]. Recently, the COG designed a prospective study to determine if the addition of i.v. gammaglobulin to chemotherapy and steroids would improve the neurologic outcome for patients with NB tumors and coincident OMS.

**NEW THERAPEUTIC AGENTS**

**Cytotoxic Agents**

Responses to topotecan, a topoisomerase I inhibitor, have been observed in patients with refractory or recurrent NB [113]. Combination therapy with topotecan plus cyclophosphamide or carboplatin has also been shown to have activity against recurrent NB [114, 115]. These promising results led to the development of a COG pilot study designed to test the clinical efficacy of incorporating topotecan into an intensive induction regimen in newly diagnosed high-risk NB patients. The clinical efficacy of another topoisomerase I inhibitor, irinotecan, is currently being tested in COG phase I trials.

**Retinoids**

Retinoids are natural and synthetic derivatives of vitamin A. Treatment with all-trans-RA (ATRA) and 13-cis-RA induces NB differentiation in vitro, downregulates MYCN mRNA expression, and leads to a sustained arrest of tumor cell proliferation [116-119]. These laboratory observations prompted the development of clinical trials designed to test the clinical utility of 13-cis-RA in children with relapsed NB. In phase I and II trials, the overall activity of 13-cis-RA in patients with high tumor burdens was disappointing [120, 121]. However, as mentioned above, in a subsequent randomized phase III study, 13-cis-RA was shown to improve the 3-year EFS rate when it was administered to patients with minimal residual disease following completion of consolidation therapy (Fig. 4) [11]. Thus, 13-cis-RA appears to be most effective in the setting of minimal residual disease.

The synthetic retinoid fenretinide (4-HPR) has also been shown to inhibit NB growth in vitro, and it is highly active against RA-resistant NB cell lines [117]. In contrast to 13-cis-RA and ATRA, 4-HPR induces apoptosis and necrosis [122]. Furthermore, a recent report indicates that 4-HPR may also inhibit NB-induced angiogenesis [123]. Phase I and II trials are being conducted to determine the clinical activity of this compound against NB.

**Immunotherapy**

Another promising approach for the treatment of multidrug-resistant microscopic disease is targeted immunotherapy, which exploits tumor selectivity and has minimal cross-resistance and overlapping toxicities with chemotherapy. Disialoganglioside (GD2) is a particularly suitable target for immunotherapy because this antigen is expressed at a high density in the majority of human NB tumors [124]. Several anti-GD2 monoclonal antibodies have been developed and tested in clinical trials [125]. Initial studies were performed with murine monoclonal antibodies (3F8 and 14G.2a), and more recently, a human-murine chimeric antibody (ch14.18) was developed and tested [126]. Therapeutic responses have been observed in phase I and phase II studies with both the murine and chimeric antibodies [127-130]. In small series of patients, responses have been reported with radiolabelled 131I-3F8 antibody (8-28 mCi/kg) followed by autologous bone marrow rescue [126, 131].

In an effort to enhance response rates, cytokines have been combined with anti-GD2 antibodies to increase antibody-dependent cellular cytotoxicity (ADCC). GM-CSF has been shown to increase leukocyte number and enhance their anti-GD2 mediated ADCC [132], and therapeutic responses have been observed in trials using ch14.18 anti-GD2 antibody plus GM-CSF in patients with recurrent/refractory NB [125]. Interleukin-2 (IL-2) has also been shown to augment lymphocyte-mediated ADCC [133]. Enhancement of natural killer cell activity by IL-2 was observed in some patients treated with the combination of anti-GD2 and IL-2 [134, 135]. The COG is currently conducting a randomized phase III trial that has been designed to determine if the addition of ch14.18 anti-GD2 antibody and cytokines to 13-cis-RA in the setting of minimal residual disease will improve the outcome of high-risk NB patients.
Other targeted immunotherapy studies are being conducted with cytokine-modified NB cells, cytotoxic T lymphocytes, modified dendritic cells, and recombinant IL-2 [136-139]. IL-2 has been infused following myeloablative therapy and stem cell rescue in several small series [140-142]. To target delivery of cytokine therapy and achieve more effective concentrations of IL-2 in the tumor microenvironment, a ch14.18-IL-2 fusion protein has recently been generated [143]. Preclinical studies have demonstrated that this fusion protein induces a cellular immune response that results in inhibited tumor growth. A COG phase I study testing the ch14.18-IL-2 fusion protein in relapsed NB patients is ongoing.

**Radioiodinated mIBG**

Radioiodinated mIBG has been used to target delivery of radiotherapy, and responses have been observed [144, 145]. Promising results have also been reported with combination radiolabelled mIBG and myeloablative chemotherapy followed by autologous stem cell rescue [146]. Additional clinical trials are ongoing in North America and Europe that will hopefully determine the optimal dose, schedule, and timing of mIBG therapy.

**Antiangiogenic Therapy**

Angiogenesis plays an important role in the growth and metastasis of malignant tumors [147]. In NB, high-level expression of angiogenesis activators and high tumor vascularity have been shown to correlate with advanced-staged disease, whereas low vascular tumor density correlates with localized disease and favorable outcome [148, 149]. Furthermore, preclinical studies have demonstrated that antiangiogenic agents effectively inhibit NB growth in vivo and that the optimal response may be in the setting of minimal residual disease [150-154]. These observations suggest that angiogenesis inhibitors may be effective in the treatment of patients with highly vascular NB tumors. Phase I studies testing a number of angiogenesis inhibitors are ongoing.

**Late Effects of Therapy**

Although intensive multimodality treatment has resulted in improved survival of children with various types of cancer, including high-risk NB, follow-up of these survivors has uncovered notable adverse long-term sequelae related to treatment [24]. These so-called “late effects” have diverse manifestations that can significantly impair quality of life and lead to a greater rate of premature mortality [155, 156]. Because of the complex nature of these late effects, it is imperative to follow cancer survivors in a comprehensive clinic that has a clear emphasis on these long-term issues. Musculoskeletal abnormalities, including scoliosis, osteoporosis, and bony and soft tissue hypoplasia, may occur as a result of surgery and/or radiotherapy. The chemotherapeutic regimens used in the treatment of NB include many agents with known long-term toxicities. Cardiopulmonary sequelae can result from anthracyclines or thoracic radiotherapy [157-159]. Otoxicity is a well-established toxicity of cisplatin. Renal-tubule-damaging chemotherapy, including ifosfamide and cisplatin, may lead to chronic electrolyte abnormalities, and these symptoms may be further exacerbated by abdominal radiotherapy and nephrectomy [159, 160]. Alkylating agents and radiation clearly impair gonadal function, and thereby, negatively impact sexual maturation and fertility [159, 161]. Other endocrine consequences of intensive multimodality anticancer therapy include growth hormone deficiency, premature menopause, thyroid and pituitary dysfunction, and obesity [159, 161, 162]. Late effects of chemotherapy and radiation also include second malignant neoplasms. Treatment-related myelodysplasia and leukemia have been seen in NB patients following dose-intensive therapy [96, 163]. Solid tumors are also observed in NB patients, and one study indicated that NB was the most common first neoplasm among 23 patients with radiation-induced thyroid cancer [164]. Some childhood cancer survivors also suffer chronically from the psychological effects of cancer, its treatment, and treatment-related cosmetic, functional, or other consequences [161]. As the outcomes of high-risk NB patients improve, it becomes increasingly important to develop new therapeutic strategies that will lead to higher rates of survival as well as enhanced quality of life.

**Conclusion**

Although substantial progress has been made in the treatment of children with low- and intermediate-risk NB, cure rates for high-risk patients remain low. Research aimed at discovering new genes and pathways critical to NB tumorigenesis and drug resistance should be prioritized in an effort to identify new targets for therapeutic strategies. Hopefully, further development of innovative, biologically based treatment approaches will prove to be effective in the treatment of NB and result in improved survival of children with clinically aggressive NB.

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