Neuroblastoma
Paradigm for Precision Medicine

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INTRODUCTION

Neuroblastoma (NB), the most common extracranial tumor of childhood, is a cancer of primordial neural crest cells that give rise to sympathetic neural ganglia and adrenal medulla. NB has a diverse pattern of clinical presentation and prognosis that ranges from low-risk to high-risk disease. Clinical and biological prognostic factors are used to risk stratify patients into groups with low, intermediate, and high risk for recurrence; most protocols now use the International Neuroblastoma Risk Group classification system. Age, stage, histology, and amplification of the MYCN oncogene are currently the most robust prognostic factors. Outcomes for low- and intermediate-risk NB are excellent, but survival for high-risk NB is less than 50%.

High-risk NB tumors contain many segmental chromosome aberrations (eg, loss of heterozygosity 1p, 11q); but recurrent somatic mutations are rare, with anaplastic lymphoma kinase (ALK) being the most commonly altered gene in approximately 10% of NB. Survival after relapse of metastatic NB is uncommon; current and upcoming trials will rely on incorporation of novel immunotherapies, inhibitors of aberrant pathways (eg MYC, ALK), and radioisotope-containing regimens, such as high-dose iodine-131-metaiodobenzylguanidine.

KEYWORDS
- Neuroblastoma
- Risk stratification
- MYCN
- Segmental chromosome aberrations (SCA)
- ALK (anaplastic lymphoma kinase)
- Phox2B
- Myeloablative therapy (MAT)
- Immunotherapy

KEY POINTS
- Neuroblastoma (NB) is the most common extracranial pediatric tumor, most frequently diagnosed cancer in infancy, and has a heterogeneous presentation and prognosis.
- Clinical and biological prognostic factors are used to risk stratify patients into groups with low, intermediate, and high risk for recurrence; most protocols now use the International Neuroblastoma Risk Group classification system.
- Age, stage, histology, and amplification of the MYCN oncogene are currently the most robust prognostic factors.
- Outcomes for low- and intermediate-risk NB are excellent, but survival for high-risk NB is less than 50%.
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- Survival after relapse of metastatic NB is uncommon; current and upcoming trials will rely on incorporation of novel immunotherapies, inhibitors of aberrant pathways (eg MYC, ALK), and radioisotope-containing regimens, such as high-dose iodine-131-metaiodobenzylguanidine.

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from spontaneous regression to aggressive metastatic tumors. For more than 2 decades, NB treatment has served as a paradigm for the incorporation of clinical and biological factors to stratify patients and tailor therapies. Using clinical, pathologic, and increasingly genetic factors, patients can be categorized as low, intermediate (IR), and high risk (HR) for recurrence. The overall survival (OS) for patients with low and IR NB is excellent at greater than 90% with relatively minimal surgical or medical interventions (Fig. 1). The goal of recent trials for non-HR patients has been to decrease treatments further and minimize chemotherapy-related toxicities. In contrast, long-term survival for HR patients remains 40% to 50% despite intensification of treatments and incorporation of immunotherapies. Current protocols are aimed at identifying better predictors of response and outcome as well as discovering genetic aberrations that may represent tractable therapeutic targets. This article summarizes the clinical presentations and current understanding of NB biology and prognostic features, their roles in risk stratification–based treatments, and novel therapies for patients with recurrent disease.

EPIDEMIOLOGY AND GENETIC PREDISPOSITION

The incidence of NB in North America and Europe is 10.5 per million children between 0 and 14 years of age, with a slight male predominance (1.2:1.0). NB is the most common cancer diagnosed in infancy, with most patients diagnosed between 0 and 4 years of age (median age 19 months), and less than 5% at greater than 10 years. NB accounts for 8% to 10% of all pediatric cancers and 12% to 15% of cancer-related deaths in children. Although there are no significant geographic variations in incidence, there are ethnic disparities in outcome. African American and Native American patients are more likely to have HR features and poor outcomes, in part because of genetic differences. Environmental factors, including parental exposures, have not been clearly linked with NB development.
NB is the only solid tumor of childhood for which there have been large screening initiatives, pioneered largely in Japan. Universal screening of 6-month-old asymptomatic infants by detection of elevated urinary catecholamines resulted in a 2-fold increase in NB incidence to 20.1 per million children; however, most of the detected tumors had favorable clinical and biological characteristics. Studies in Germany and Quebec also demonstrated an increased incidence and detection of tumors with favorable biology and pathology. In general, universal screening has not detected poor prognosis disease, which usually presents at an older age and, thus, has not affected mortality rates. In contrast, in selected populations with an inherited genetic predisposition to NB, screening may be indicated.

Genetic Predisposition

The incidence of familial NB is estimated at 1% to 2%. Cases often involve multifocal and/or bilateral adrenal primary tumors with a median age of onset of 9 months. The pattern of inheritance is autosomal dominant with incomplete penetrance. NB can occur in patients with other neural crest disorders, such as Hirschsprung disease (HSCR), congenital central hypoventilation syndrome (CCHS), and neurofibromatosis type 1 (NF1). Mutations in the Phox2b homeobox gene have been detected in subsets of patients with familial NB and usually are associated with other neurocristopathies, such as HSCR and CCHS. Phox2b mutations have also been detected in approximately 2% of sporadic NB. There are many reports of NB in patients with NF; however, there are conflicting data as to whether germline NF1 mutations are associated with an increased risk to develop NB.

Linkage studies in familial NB pedigrees identified candidate chromosomal predisposition regions including 16p, 12p, and 2p and led to the identification of germline mutations in the tyrosine kinase domain of the anaplastic lymphoma kinase (ALK) oncogene. ALK is involved in nervous system development, and central nervous system (CNS) anomalies have been reported in some patients with germline ALK mutations. Sporadic NB tumors also harbor ALK abnormalities, including genomic amplification (2%–3%) and missense mutations (8%–12%). Studies of ALK inhibitors in NB and other tumors with ALK aberrations (eg, anaplastic large cell lymphoma) have shown promising results. NB cases are also detected in other familial cancer syndromes, including Beckwith-Wiedemann syndrome, Li-Fraumeni, Noonan (PTPN11), some subtypes of Fanconi anemia, and some chromosomal breakage syndromes.

Recent genome-wide association studies using peripheral blood from thousands of patients with NB have also identified germline genetic variants that may predispose to the development of sporadic NB. These variants include single nucleotide polymorphisms (SNPs) in LINC00340, BARD1, LMO1, DUSP12, DDX4, LIN28B, HACE1, and TP53. Unlike the rare germline mutations in ALK and Phox2B described earlier, these SNPs are more frequent but individually have less dramatic impacts on the NB risk. The interplay between multiple germline variants and somatic alterations, discussed later, may influence the initiation and progression of NB.

PRESENTATION, DIAGNOSIS, AND STAGING

Symptoms

NB presentations vary based on the disease extent and tumor location, which may occur anywhere along the sympathetic chain resulting in local effects on organs, vessels, or nerves (Fig. 2, Table 1). Most of them (65%) arise in the abdomen, most...
commonly the adrenal gland, and may be asymptomatic or associated with hypertension, abdominal pain, distension, and constipation. Other sites include the neck, chest, and pelvis. The primary site location is associated with age and outcome. Cervical and thoracic tumors are more common in infants and may present with Horner syndrome (unilateral ptosis, anhidrosis, and myosis) and respiratory 

**Table 1**

<table>
<thead>
<tr>
<th>Location</th>
<th>Signs and Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen/pelvis</td>
<td>Pain, constipation, distension, urinary retention, hypertension</td>
</tr>
<tr>
<td>Thorax</td>
<td>Respiratory distress, Horner syndrome</td>
</tr>
<tr>
<td>Presacral and paraspinal (includes abdominal and thoracic masses)</td>
<td>Symptoms of cord compression (urinary retention, paraplegia/paraparesis, clonus)</td>
</tr>
<tr>
<td>Neck</td>
<td>Mass/swelling</td>
</tr>
<tr>
<td>Metastases</td>
<td>Irritability, bone pain, cytopenias (petechiae, ecchymoses, pallor), periorbital ecchymoses, fever, weight loss, lymphadenopathy</td>
</tr>
<tr>
<td>4S/4M metastases</td>
<td>Hepatomegaly, coagulopathy, hyperbilirubinemia, respiratory distress (from abdominal enlargement), skin nodules</td>
</tr>
<tr>
<td>Paraneoplastic syndromes</td>
<td>• OMS: myoclonic jerking and random eye movement, with or without cerebellar ataxia</td>
</tr>
<tr>
<td></td>
<td>• VIP secreting tumors: intractable secretory diarrhea caused by tumor secretion of VIP</td>
</tr>
</tbody>
</table>

Patients may be asymptomatic or may have one or more of the listed symptoms or findings on exam. 

*Abbreviations*: OMS, opsoclonus myoclonus ataxia syndrome; VIP, vasoactive intestinal peptide.
symptoms. Epidural or intradural tumor extension occurs in 5% to 15% of patients and may result in spinal cord compression and paraplegia.\(^\text{51}\) Two rare paraneoplastic syndromes associated with NB include secretory diarrhea caused by tumor production of vasoactive intestinal peptide\(^\text{52,53}\) and opsoclonus myoclonus ataxia syndrome (OMS). OMS is reported in 2% to 3% of patients and is commonly associated with favorable well-differentiated tumors.\(^\text{54,55}\) OMS is characterized by myoclonic jerks and random eye movements with or without ataxia, is attributed to immune-mediated effects, and often persists after resection, resulting in significant neurodevelopmental sequelae.

Approximately half of patients present with localized or regional disease, and 35% have regional lymph node spread at the time of diagnosis. Distant metastases are detected in 50% of patients at diagnosis and occur through both lymphatic and hematogenous routes. The most common sites include bone, bone marrow, and liver. NB has a particular predilection to spread to metaphyseal, skull, and orbital bone sites, resulting in a classic presentation characterized by periorbital ecchymoses (raccoon eyes), proptosis, and potentially visual impairment. In contrast to the frequent lack of symptoms for locoregional tumors, patients with widespread disease are often ill appearing with fever, pain, irritability, and weight loss. Less common sites of metastases at diagnosis include the lung\(^\text{56}\) and brain; however, CNS disease at relapse is increasingly common.\(^\text{57,58}\) In infants there is an unusual pattern of metastases, stage 4S or MS (see “Staging” later), characterized by skin nodules and/or diffuse liver involvement and hepatomegaly often associated with respiratory compromise.\(^\text{59}\)

Diagnosis is confirmed either by (1) tumor tissue biopsy and histopathology (Fig. 3) or (2) a combination of NB tumor cells detected in bone marrow together with elevated urine or serum catecholamine or catecholamine metabolites (dopamine, vanillylmandelic acid, and homovanillic acid). Evaluation includes cross-sectional imaging with computed tomography or MRI to determine size, regional extent (including intraspinal invasion), distant spread to neck, thorax, abdomen and pelvis (see Fig. 4).\(^\text{60,61}\) Bilateral iliac crest bone marrow aspirates and biopsies are required to determine tumor involvement by histology. Radioiodine-labeled metaiodobenzylguanidine (MIBG), a norepinephrine analogue that selectively concentrates in sympathetic nervous tissue, is used to detect primary tumors and metastatic sites.\(^\text{62}\) Approximately 90% of patients have MIBG-avid disease, and semiquantitative scoring systems are being integrated into NB response criteria.\(^\text{63,64}\) [(18)F-fluorodeoxyglucose positron emission tomography (FDG-PET) scans are recommended for detecting metastatic disease in patients whose tumors are not MIBG avid.\(^\text{65-67}\) Technetium bone scans can be used to detect cortical bone disease if MIBG and PET scan are not available.

**Staging**

Until recently, the criteria for diagnosis and staging were based on the surgical-pathologic International Neuroblastoma Staging System (INSS) (Box 1).\(^\text{68,69}\) INSS stages 1 to 3 are localized tumors that are classified based on the amount of resection, local invasion, and node involvement. Stage 4 is defined as distant metastases; 4S (4Special) is characterized by metastases to the liver, skin, and/or marrow in infants, which is usually associated with favorable biological features and can undergo spontaneous regression. In 2009, the International Neuroblastoma Risk Group’s (INRG) stratification system was developed by representatives from a major consortium in North America (Children’s Oncology Group [COG]) Europe (SIOPEN, International Society of Pediatric Oncology European Neuroblastoma), and Germany, Japan, and Australia. The INRG staging system (INRGSS) uses surgical risk factors (SRFs), which are preoperative radiological features to distinguish locoregional tumors that do not
involves local structures (INRG L1) from locally invasive tumors with imaging-defined risk factors (IDRFs) (INRG L2) (Boxes 2 and 3).\textsuperscript{70,71} INRGS M and MS refer to tumors with distant metastases and have the INSS 4 or 4S pattern of spread, respectively.

**Fig. 3.** Histopathology and fluorescence in situ hybridization (FISH) assays (A–C). Shown are representative images (hematoxylin-eosin, original magnification \( \times 200 \) [A and C] \( \times 400 \)) from 3 different histologic appearances of NB: (A) poorly differentiated NB, (B) differentiating NB, and (C) ganglioneuroblastoma (stroma-rich NB). The fluffy pink material separating the cells is neuropil (categorized as stroma-poor). (A) The poorly differentiated NB cells have minimal cytoplasm, discernible only as purple-stained nuclei. (B) The neuroblasts are differentiating as reflected by defined pink cytoplasm and larger nuclei. (C) The neuroblasts have the features of fully differentiated ganglion cells, and the spindle cell areas in the 4 corners are composed of Schwann cells (categorized as stroma-rich). (D) FISH showing MYCN amplification (MYCNA). The presence of multiple copies of MYCN is detected in tumor cells using a labeled probe (red) for the chromosomal location 2p region that includes the MYCN gene. MYCNA is defined as greater than 10 copies. (E) FISH showing 1p loss of heterozygosity (LOH). Cells show 2 signals from the control 1q probe (green) and 1 signal for the 1p 36 probe (red) indicating that there is loss of one copy of 1p36 loci (LOH) and 2 normal copies of 1q. (Courtesy of Dr Paul Thorner, Pathology Department, and Dr Mary Shago, Cytogenetics Laboratory, Hospital for Sick Children, Toronto.)

CLINICAL AND BIOLOGICAL RISK FACTORS, PROGNOSIS, AND RISK STRATIFICATION

NB is classified into low risk, IR, and HR based on clinical and biological factors that have been shown to predict prognosis and risk of recurrence, including age, stage, histopathology, DNA index (ploidy), and MYCN amplification (MYCNA) and are used to assign treatment (Table 2). In comparison, the recently developed INRG classification system defines similar cohorts using the INRG database (8800 patients treated between 1990–2002) to facilitate comparisons across international clinical trials (Box 4).\textsuperscript{70}
Fig. 4. Diagnostic imaging of NB. Shown are representative images of NB tumors from different primary locations from diagnostic evaluations. (A) Computed tomography (CT) scan (axial view) shows a typical retroperitoneal mass arising from the adrenal with calcifications (white speckles in tumor mass, black arrows) and tumor encasement of vessels (aorta, white arrow). The left kidney demonstrates mild pelviectasis, which is commonly seen secondary to the mass effect. (B) CT scan (coronal view) of very large liver with multiple NB tumor nodules (darker than surrounding liver parenchyma), which is typically seen in infants with International Neuroblastoma Staging System stage 4S/INRG MS. (C) MRI scan (sagittal view) shows a paraspinal thoracic mass (arrows) with intraspinal extension and spinal cord compression. (D) Brain and orbital CT (axial) with large metastases involving the orbits, with more extensive involvement on the left (arrow). (E) I-123 metaiodobenzylguanidine scan demonstrates widespread bony metastases in the extremities, vertebrae, and pelvis (darker lesions). Note the normal physiologic uptake in the heart, liver, and bladder.
Many studies have consistently demonstrated the independent prognostic value of the INSS stage, including an INRG database analysis that reported superior event-free survival (EFS) and OS for patients with nonmetastatic NB (INSS stages 1, 2, and 3) and INSS stage 4S (83 ± 1% and 91 ± 1%) compared with only 35 ± 1 and 42 ± 1%, for patients with INSS stage 4 disease. With the exception of stage 4S, specific metastatic sites have not been incorporated into staging systems. However, retrospective studies suggest that spread confined to distant lymph nodes may predict improved outcomes, whereas there is a trend toward inferior outcomes for patients with metastases to the lung or bone marrow. Although retrospective studies have demonstrated the prognostic significance of the INRGSS, which incorporates SRFs, this will be prospectively validated across North America and Europe.

Age was one of the first prognostic indicators identified. In comparison to infants, patients older than 1 to 2 years at diagnosis have an inferior outcome; this effect is more prominent for patients with metastatic disease. Historically, a cutoff of 365 days had been used as a surrogate for tumor behavior; however, London and

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**Stage and Age**

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### International Neuroblastoma Staging System (INSS)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</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 resection; 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 for 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 y of age)</td>
</tr>
</tbody>
</table>

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*Box 1: International Neuroblastoma Staging System (INSS)*

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colleagues studied the continuous nature of age for 3666 patients and concluded that the most prognostic cutoff was 460 days (15.1 months). Several retrospective studies specifically examined whether 18 months might represent a more clinically relevant cutoff and demonstrated that EFS and OS for patients with INSS stage 4 disease aged 12 to 18 months (with favorable tumor biology) was similar to that of patients aged less than 12 months. Similarly, patients with INSS stage 3 disease aged 12 to 18 months had a superior outcome to those older than 18 months. Prospective COG trials will determine whether reduction of therapy for toddlers aged 12 to 18 months with biologically favorable tumors, traditionally treated with more intensive regimens, will still provide superior outcomes. Older children, adolescents, and young adults with NB have a more indolent course and worse overall outcome despite infrequent MYCN oncogene amplification (MYCNA); however, no specific prognostic age cutoffs greater than 18 months have been identified.

**Histopathology**

Pathologic characteristics have been used to further classify tumors into favorable and unfavorable categories, initially using a system developed by Shimada and colleagues that provided the basis for the more recently revised International Neuroblastoma Pathology Committee (INPC) criteria. The prognostic value of INPC classification, based on age, presence of Schwannian stroma, grade of neuroblastic differentiation, and Mitosis-karyorrhexis index, has been validated in large cooperative group studies to identify specific patient risk groups that may benefit from modified therapy. In the COG P9641, patients with INSS stage 1 and 2 disease with favorable histology had a significantly better outcome than those with unfavorable histology (UH) (EFS 90 ± 3% and 72 ± 7%, OS 99 ± 1% and 86 ± 5%).

**Tumor Genetics**

NB genetic features have been used for risk stratification for more than 20 years. Two broad categories of genetic aberration patterns include (1) tumors with whole chromosome gains, lack of structural changes, and hyperdiploid karyotype and (2) tumors with segmental chromosomal aberrations (SCAs) and diploid DNA content, which are often associated with poor outcomes. SCAs often include partial gains and losses of chromosomal regions predicted to encode oncogenes and tumor suppressors, respectively.

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**Box 2**

**International Risk Group Staging System (INRGSS)**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Localized tumor not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment</td>
</tr>
<tr>
<td>L2</td>
<td>Locoregional tumor with presence of one or more IDRFs (see Box 1)</td>
</tr>
<tr>
<td>M</td>
<td>Distant metastatic disease (except stage MS)</td>
</tr>
<tr>
<td>MS</td>
<td>Metastatic disease in children younger than 18 mo with metastases confined to skin, liver, and/or bone marrow</td>
</tr>
</tbody>
</table>

Patients with multifocal primary tumors should be staged according to the greatest extent of disease as defined in the table.

**Allelic Gains, Amplifications, and Oncogenes**

*MYCNA* defined as greater than 10 copies\(^8^3\) is detected in approximately 20% of NB tumors, with a higher incidence in INSS stages 3 and 4 (40%) but only 5% of stages 1, 2, and 4s.\(^7^0\) Many studies have demonstrated that in comparison to patients with non-*MYCNA* tumors, patients with *MYCNA* have a significantly worse outcome\(^8^4,^8^5\) (reviewed in Refs.\(^4^9,^8^6,^8^7\)). All patients with *MYCNA* stage 3, 4 and 4S tumors are classified as HR, including infants; however, the prognostic significance of *MYCNA* in rare cases of localized resected NB remains controversial.\(^8^8,^8^9\) Importantly, most laboratory animal models for NB rely on overexpression of MYCN in neural crest cells\(^3^6,^9^0\); recent studies have identified drugs that target MYCN to inhibit NB growth.\(^9^1\)

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**Box 3**

<table>
<thead>
<tr>
<th>Image Defined Risk Factors (IDRFs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ipsilateral tumor extension within 2 body compartments</strong></td>
</tr>
<tr>
<td>Neck-chest, chest-abdomen, abdomen-pelvis</td>
</tr>
<tr>
<td><strong>Neck</strong></td>
</tr>
<tr>
<td>Encases carotid and/or vertebral artery and/or internal jugular vein; extends to skull base; compresses trachea</td>
</tr>
<tr>
<td><strong>Cervicothoracic junction</strong></td>
</tr>
<tr>
<td>Encases brachial plexus roots or subclavian vessels and/or vertebral or carotid artery; compresses trachea</td>
</tr>
<tr>
<td><strong>Thorax</strong></td>
</tr>
<tr>
<td>Encases the aorta and/or major branches; compresses trachea and/or principal bronchi; lower mediastinal tumor infiltrating costovertebral junction between T9 and 12</td>
</tr>
<tr>
<td><strong>Thoracoabdominal</strong></td>
</tr>
<tr>
<td>Encases the aorta and/or vena cava</td>
</tr>
<tr>
<td><strong>Abdomen/pelvis</strong></td>
</tr>
<tr>
<td>Infiltrates the porta hepatis and/or the hepatoduodenal ligament; encases branches of the superior mesenteric artery at the mesenteric root or origin of celiac axis and/or superior mesenteric artery; invades one or both renal pedicles; encases aorta and/or vena cava or iliac vessels, crossing sciatic notch</td>
</tr>
<tr>
<td><strong>Intraspinal tumor extension whatever the location provided that</strong></td>
</tr>
<tr>
<td>More than one-third of the spinal canal in the axial plane invaded and/or the perimedullary leptomeningeal spaces not visible and/or the spinal cord signal abnormal</td>
</tr>
<tr>
<td><strong>Infiltration of adjacent organs/structures</strong></td>
</tr>
<tr>
<td>Pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, and mesentery</td>
</tr>
</tbody>
</table>

**Conditions to be recorded but not considered IDRFs**

- Multifocal primary tumors
- Pleural effusion, with or without malignant cells
- Ascites, with or without malignant cells

IDRFs are used to determine the ability to completely resect locoregional tumors at diagnosis based on surgical risk factors that can be defined by IDRFs detected on cross-sectional imaging with CT and/or MRI.

Amplification of ALK, located at 2p23 in close proximity to MYCN at 2p24, is detected in 2% to 3% of NB and is more common, though not exclusively, observed in tumors with MYCN amplification. A 17q gain detected in greater than 60% of tumors is often associated with other poor prognostic markers (eg, MYCNA, older age). Although the specific genes that may have oncogenic roles on 17q have not been identified, candidates include BIRC5 (survivin), PPMID (WIP1), and NME1/2.

Allelic Losses and Tumor Suppressor Genes

The most frequently deleted chromosomal regions in NB include 1p, 4p, 11q, and 14q. Chromosome 1p loss of heterozygosity (LOH), detected in 23%–30% of tumors, predicts poor outcome. 1p36 LOH correlates with MYCNA and other HR features (eg, metastasis, age >1, UH), and thus, 1pLOH may be most relevant as an independent prognostic factor in infants and patients without MYCNA. The 1p candidate tumor suppressor genes include the chromatin remodeling protein CHD5 and transcription factor CASZ1. Chromosome 11qLOH is detected in approximately 30% to 40% of patients. Like 17q gain and 1pLOH, 11qLOH is more common in patients with stage 4 disease and predicts poor prognosis; however, 11qLOH is rarely associated with MYCNA and, therefore, may predict additional HR subsets within the non-MYCNA tumors. One 11q candidate gene, CADM1, has been implicated in NB growth and proliferation. Although INRG currently includes 11qLOH as criteria to upstage to

Table 2: Children's Oncology Group Neuroblastoma Risk Stratification

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Stage</th>
<th>Age</th>
<th>MYCN</th>
<th>Ploidy</th>
<th>Shimada</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk 1</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
</tr>
<tr>
<td>Low risk 2a/2b</td>
<td>Any</td>
<td>Not amp</td>
<td>Any</td>
<td>Any</td>
<td></td>
</tr>
<tr>
<td>HR 2a/2b</td>
<td>Any</td>
<td>Amp</td>
<td>Any</td>
<td>Any</td>
<td></td>
</tr>
<tr>
<td>IR 3</td>
<td>&lt;547 d</td>
<td>Not amp</td>
<td>Any</td>
<td>Any</td>
<td></td>
</tr>
<tr>
<td>IR 3</td>
<td>&gt;547 d</td>
<td>Not amp</td>
<td>Any</td>
<td>FH</td>
<td></td>
</tr>
<tr>
<td>HR 3</td>
<td>Any</td>
<td>Amp</td>
<td>Any</td>
<td>Any</td>
<td></td>
</tr>
<tr>
<td>HR 4</td>
<td>&lt;365 d</td>
<td>Amp</td>
<td>Any</td>
<td>Any</td>
<td></td>
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<tr>
<td>IR 4</td>
<td>&lt;365 d</td>
<td>Not amp</td>
<td>Any</td>
<td>Any</td>
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<tr>
<td>HR 4</td>
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<td>Amp</td>
<td>Any</td>
<td>Any</td>
<td></td>
</tr>
<tr>
<td>HR 4</td>
<td>365–&lt;547 d</td>
<td>Any</td>
<td>Dl=1</td>
<td>FH</td>
<td></td>
</tr>
<tr>
<td>IR 4</td>
<td>365–&lt;547 d</td>
<td>Any</td>
<td>Dl=1</td>
<td>FH</td>
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<td>Low risk 4s</td>
<td>&lt;365 d</td>
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<td>Dl=1</td>
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<td>HR 4s</td>
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<td>Not amp</td>
<td>Any</td>
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</tr>
</tbody>
</table>

COG currently uses the International Neuroblastoma Staging System’s stage, age, MYCN status, DNA index or ploidy, and INPC histology to determine patient’s risk category as high, intermediate or low.64

Abbreviations: amp, amplification; Dl, DNA index; FH, favorable histology; UH, unfavorable histology.

<table>
<thead>
<tr>
<th>INRG Stage</th>
<th>Age (mo)</th>
<th>Histologic Category</th>
<th>Grade of Tumor Differentiation</th>
<th>MYCN Aberration</th>
<th>Ploidy</th>
<th>Pretreatment Risk Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1/L2</td>
<td>—</td>
<td>GN maturing; GNB intermixed</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>A. Very low</td>
</tr>
<tr>
<td>L1</td>
<td>—</td>
<td>Any, except GN maturing or GNB intermixed</td>
<td>—</td>
<td>NA</td>
<td>—</td>
<td>B. Very low</td>
</tr>
<tr>
<td>L2 &lt;18</td>
<td>Any, except GN maturing or GNB intermixed</td>
<td>—</td>
<td>NA</td>
<td>No</td>
<td>—</td>
<td>D. Low</td>
</tr>
<tr>
<td>≥18</td>
<td>GNB nodular; neuroblastoma</td>
<td>Differentiating</td>
<td>NA</td>
<td>No</td>
<td>—</td>
<td>E. Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poorly differentiated or undifferentiated</td>
<td>NA</td>
<td>—</td>
<td>—</td>
<td>H. Intermediate</td>
</tr>
<tr>
<td>M &lt;18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Amp</td>
<td>Hyperdiploid</td>
<td>F. Low</td>
</tr>
<tr>
<td>12–&lt;18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>NA</td>
<td>—</td>
<td>I. Intermediate</td>
</tr>
<tr>
<td>&lt;18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>NA</td>
<td>—</td>
<td>J. Intermediate</td>
</tr>
<tr>
<td>≥18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Amp</td>
<td>—</td>
<td>O. High</td>
</tr>
<tr>
<td>MS &lt;18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Amp</td>
<td>R. High</td>
</tr>
</tbody>
</table>

Classification schema is based on analysis of 8800 patients in the INRG database (1990–2002). Risk groups are very low risk (5-year event-free survival [EFS] >85%); low risk (5-year EFS >75% to ≤85%); IR (5-year EFS ≥50% to ≤75%); HR (5-year EFS <50%).

Staging of L1, L2, M, and MS described in Fig. 2B.

Abbreviations: Amp, amplified; EFS, event-free survival; GN, ganglioneuroma; GNB, ganglioneuroblastoma; NA, not amplified.

HR classification, prospective trials are ongoing to determine whether 11qLOH predicts poor outcomes for non-HR patients.

**Segmental Chromosome Aberrations**

Historically, individual chromosomal loci were analyzed using polymerase chain reaction or fluorescent in situ hybridization–based assays. Recent studies using techniques that assess the whole genome, such as comparative genome hybridization and SNP arrays, demonstrate that it is the genomic pattern and not individual losses/gains that is most prognostic. Tumors with numerical chromosomal abnormalities (NCAs) characterized by whole chromosome gains and losses have an excellent outcome, even in patients greater than 18 mo. In contrast, patients with segmental chromosome aberrations (SCA), characterized by gains and losses of smaller fragments, have an inferior outcome.\(^{102,103}\) SCAs may be a particularly strong predictor of poor outcome in infants with locally unresectable or metastatic non-MYCN amplified tumors.\(^{104}\) Prospective trials in North America and Europe will determine whether the presence of SCAs (>1 of the following: segmental loss at 1p, 3p, 4p, 11q or gain at 1q, 2p, or 17q) can distinguish less favorable subsets of patients within the non-HR groups of patients and potentially replace tests that detect single gene losses/gains.

**DNA Content**

Ploidy or tumor DNA content (chromosome number) is a powerful predictor of survival. Hyperdiploid tumors (DNA index >1) with an increased amount of DNA in comparison with diploid tumors (DNA index = 1) are associated with a more favorable prognosis.\(^{105,106}\) Ploidy is most prognostic in infants and patients with localized disease\(^{74,107}\) and has been used prospectively to inform risk assignment and tailor therapy for patients with non-HR NB.\(^{108}\)

**Somatic Gene Mutations**

Recently, next-generation sequencing approaches have revealed that, in contrast to adult carcinomas, there is a striking lack of recurrent NB tumor (somatic) mutations.\(^{109–112}\) The most commonly mutated gene is ALK (8%–10%), with an additional 3% harboring ALK amplification.\(^{10}\) ALK genomic aberrations are detected in all risk groups and are associated with an adverse outcome,\(^{33}\) and high levels of ALK protein or amplification may correlate with poor outcomes independent of mutation status.\(^{32,113,114}\) Mutations in ATRX (alpha thalassemia/mental retardation syndrome X linked), which is involved in telomere maintenance, are detected more frequently in older patients with NB.\(^{112}\) Deletions and point mutations of the chromatin remodeling proteins AT-rich interactive domain 1A and B (ARID1a/1b) were detected in 11% of tumors.\(^{111}\) Other mutations detected in less than 5% of tumors include MYCN, TP53, PTPN11, and genes involved in Ras/MAPK signaling. Current studies are exploring whether mutations may be more common at recurrence\(^{115}\) and whether epigenetic regulation of transcription and genomic organization, which has recently been reported to be involved in the medulloblastoma,\(^{116}\) may be playing similar roles in NB.

**Molecular Factors and Expression Signatures**

Because recurrent mutations are not frequent in NB, the identification of genes and signaling pathways with altered expression have also been used to discover additional prognostic factors and therapeutic targets involved in NB differentiation, apoptosis, drug resistance, angiogenesis, metastasis, and inflammation. Extensive reviews of these molecular factors have been the subject of several recent reviews,\(^ {49,117,118}\) and a subset of the most well studied are included later.
Neurotrophin signaling has central roles in normal neuronal cell development, and the clinical and biological roles of TRK receptors (NTRK1, 2, 3 encoding TrkA, B, C) and their ligands (NGF, BDNF, and NT-3) have been extensively studied in NB (reviewed in Ref.119). TrkA expression is highest in tumors with favorable biological characteristics and outcomes, and TrkA induces apoptosis and/or differentiation in vitro. TrkA signaling has been implicated in mediating spontaneous regression that is often observed in infants with localized or stage 4S disease. In contrast, TrkB has pro-proliferative and migratory properties, enhances chemoresistance, and is highly expressed in biologically unfavorable MYCNA NB. Although the TrkB inhibitor lestaurtinib did not show efficacy in a phase 1 trial,121 trks and proteins involved in neural crest development and differentiation pathways may still represent potential therapeutic targets.

Disruption of proteins involved in apoptotic pathways, including multidrug-resistance proteins, such as MDR-1, bcl-2 family proteins, caspase-8, mTOR/PI3 kinase, and TP53/HDM2, have also been shown to play important roles in NB initiation and progression. There are many ongoing pre-clinical studies to determine the ability to pharmacologically target these pathways. Many genes involved in NB, including caspase 8 and the RASSF1A tumor suppressor, are inactivated by the promoter hypermethylation, which contributes to resistance to apoptosis induced by many therapies. Demethylating agents, such as decitabine, have been tested in phase I studies. Enhanced angiogenesis and high expression of proangiogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor are associated with more aggressive NB tumors; early phase clinical trials of drugs that block these pathways have been completed.

Rather than focusing on specific candidate genes, several investigators have identified multigene expression profiles that predict outcome and may lead to further refinement of risk categories. One large study demonstrated that the expression of 59 genes was an independent predictor of outcome, even after controlling for currently used risk factors, with an odds ratio of 19.3 for OS and 3.96 for progression-free survival. Additional retrospective studies have identified other multigene classifiers (ranging from 3 to >50 genes). Although most of these signatures have not been studied in specific NB risk groups, Asgharzadah and colleagues recently demonstrated that a 14-gene classifier can be used to specifically identify subsets of HR patients with the worst prognosis. Many of these signatures include genes implicated in NB pathogenesis, neural development, and inflammation/immune response. Recent reports also demonstrate prognostic profiles of microRNAs, small 22 to 25 nucleotide RNAs that inhibit protein translation or target mRNA degradation (reviewed in Ref.144).

MANAGEMENT GOALS

Diagnosis and therapy requires a multidisciplinary approach. Surgical biopsy is usually required to assess tumor genetic and histologic features and is most critical for patients less than 18 months of age with metastatic disease and those with localized unresectable tumors. The improved understanding of NB biology and its impact on prognosis has resulted in successful tailoring based on risk stratification (low risk, IR, and HR) using many of the pretreatment clinical and biological risk factors discussed earlier (see Box 4, Table 2). The requirements for further surgical resection, chemotherapy, radiotherapy and/or immunotherapy is based on the patients’ specific risk category (Table 3) and, in part, response as outlined in the International Neuroblastoma Response Criteria, which is currently under revision. When
possible, exposure to chemotherapy is limited for patients with regional disease, whereas radiotherapy is limited to those with advanced disease with unfavorable characteristics.

**Low Risk**

Survival rates for patients with INSS stage 1 disease, regardless of biological factors, are excellent with surgery alone and rare recurrences can often be cured with salvage chemotherapy. Similarly, chemotherapy can be omitted for most patients with biologically favorable but incompletely resected localized tumors (INSS 2A, 2B), with survival rates greater than 95%. In general, for patients with INSS stage 1, 2A, and 2B (mostly INRG stage L1), chemotherapy is reserved for patients with localized NB who have life- or organ-threatening symptoms or the minority of patients who experience recurrence or progressive disease.

Because previous infant screening studies and European trials have suggested that subsets of biologically favorable NB can spontaneously differentiate and regress, a recent COG trial (ANBL00P2) studied whether infants less than 6 months of age with small localized adrenal masses (including those detected by prenatal ultrasound) could be observed without biopsy, surgery, or chemotherapy. Eighty-one percent of patients demonstrated spontaneous regression without surgical intervention; the 3-year EFS and OS were 97% and 100%, respectively.

Like many localized tumors in infants, most of stage 4S NB without MYCNA undergo spontaneous regression. Chemotherapy or low-dose radiotherapy is reserved for symptoms of large tumors or massive hepatomegaly causing mechanical obstruction, respiratory distress, and/or liver dysfunction and should be initiated as soon as possible to prevent the morbidity and mortality frequently associated with this form of the disease, especially in very young infants.

Overall, these data support continued reduction of chemotherapy exposure and surgery for most low risk asymptomatic patients, while strategies to improve survival for the rare subsets of non-HR patients with unfavorable pathology or biology (eg, MYCNA).

<table>
<thead>
<tr>
<th>Treatment strategies based on risk group (COG)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low (40%)</strong></td>
</tr>
<tr>
<td>Survival (EFS)</td>
</tr>
<tr>
<td>Patient/tumor characteristics</td>
</tr>
<tr>
<td>Infants with metastases (no MYCNA)</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
</tbody>
</table>

Summarized are general treatment strategies and characteristics for each risk group based on recent COG trials. This chart includes the most common characteristics for each group and overall treatment strategies. These treatments may vary across different cooperative groups internationally and change based on ongoing and future clinical trials. The approximate relative proportion of patients in each risk group is based on data from the COG ANBL00B1 Biology Study (since 2001).
diploid tumors with SCAs\textsuperscript{82,158} are being examined in prospective SIOOPEN and COG trials.\textsuperscript{64}

**Intermediate Risk**

IR classification encompasses a wide spectrum of disease for which surgical resection and moderate-dose multiagent chemotherapy are the backbone of most regimens. IR includes subsets of patients with INSS stage 3 (mostly INRG L2) disease and infants with stage 4/M disease with favorable biological features. Survival following surgical resection and moderate-dose chemotherapy, including carboplatin or cisplatin, doxorubicin, etoposide, and cyclophosphamide, is greater than 90\% for children whose tumors exhibit favorable characteristics, including infants with stage 4/M who lack MYCNA.\textsuperscript{159–161} These high survival rates were maintained in 2 prospective COG IR trials in which therapy was reduced further based on histology, ploidy, and 1p and 11q LOH status.\textsuperscript{108,157} Small series have suggested that IR patients with localized NB with favorable biology can be observed without chemotherapy.\textsuperscript{153,162} Ongoing prospective international trials will determine whether SCA status can be used to refine treatment assignment to further reduce, and in some cases eliminate, therapy for most IR patients with favorable histology and genomics.

**High Risk**

Outcome of HR patients (mainly stage 4 > 18 months of age and stage 3 MYCNA or stage 3 > 18 months with unfavorable histology tumors) remains poor despite improvements in survival (Fig. 5).\textsuperscript{163–168} Standard HR therapy involves 3 components: (1) induction chemotherapy and local control, (2) consolidation, and (3) postconsolidation/maintenance. These regimens have evolved significantly over the past 20 years based on work by several international cooperative groups and smaller cohort studies summarized later.

**Induction therapy**

There is a correlation between survival and end-of-induction response\textsuperscript{63,169}; despite chemotherapy dose intensification, approximately 20\% of patient will progress or have inadequate response to induction therapy. Standard North American (COG) induction regimens include combinations of anthracyclines, alkylators, platinum compounds, and topoisomerase II inhibitors delivered every 21 days for 5 to 7 cycles. SIOOPEN uses a rapid regimen whereby cycles are delivered every 10 days based on results that demonstrated superior 5-year EFS of 30\%, compared with 18\% for standard interval chemotherapy.\textsuperscript{165} The topoisomerase I inhibitor topotecan, which has demonstrated efficacy in recurrent NB,\textsuperscript{170} has recently been incorporated into COG induction regimens.\textsuperscript{64,171}

**Local control**

Optimal local control is achieved with a combination of aggressive surgical resection and external beam radiotherapy to the primary tumor. Surgery of the primary and bulky metastatic disease is usually delayed until after 4 to 6 cycles of chemotherapy to improve resectability and minimize complications;\textsuperscript{172} however, there are conflicting reports as to whether complete primary tumor resection impacts patient outcomes in HR NB.\textsuperscript{173–176}

NB is one of the most radiosensitive pediatric solid tumors, and doses of 2160 cGy in daily 180 cGY fractions to the primary sites decrease local recurrence rates for HR patients.\textsuperscript{177,178} A recently completed prospective COG trial will determine whether higher radiation doses delivered to incompletely resected tumors improves local control rates. Radiation is also often delivered to residual MIBG-avid metastatic sites, and
Fig. 5. Survival for HR patients with NB based on treatment era. The EFS (A) and OS (B) Kaplan-Meier survival curves calculated from the time of diagnosis for children enrolled onto COG (since 2001) and Children’s Cancer Group and Pediatric Oncology Group Neuroblastoma Biology trials between 1990 and 2010 (N = 3389) shown in 5-year intervals, beginning in 1990. (With permission from Children’s Oncology Group Statistical Data Center.)
a recent report suggests that nonirradiated lesions have a higher likelihood of involvement at the time of first relapse.\textsuperscript{179}

\textbf{Myeloablative consolidation therapy}

Over the past 2 decades, several clinical trials performed in Germany, Europe, and North America demonstrated improved outcomes following myeloablative therapy (MAT) with autologous bone marrow or, more recently, autologous peripheral blood stem cell rescue as compared with maintenance chemotherapy or observation.\textsuperscript{167,169,180,181} These data together with a recent Cochrane systems meta-analysis suggest that MAT has resulted in improvements in EFS.\textsuperscript{182} Recent and ongoing trials are aimed at identifying the optimal intensity and chemotherapy combinations for MAT regimens. Preliminary SIOPEN results suggest that patients randomized to a Busulfan-Melphalan (Bu-Mel) regimen had outcomes superior to those who received carboplatin-etoposide-melphalan.\textsuperscript{175} Before adoption of Bu-Mel, the COG and other groups are examining the efficacy and toxicities of Bu-Mel MAT in combination with different induction regimens and postconsolidation immunotherapy.\textsuperscript{64} In addition, data will soon be available from COG study ANBL0532, which randomized patients to single and tandem MAT and was based on a limited institution tandem MAT study with 3- and 5-year EFS rates of 55% and 47%.\textsuperscript{183} Future trials will also aim to identify those at highest risk for recurrence and assess whether additional therapies during induction or consolidation improve their outcome.\textsuperscript{64}

\textbf{Postconsolidation biologic and immunotherapies}

Initial results from CCG-3891 demonstrated efficacy for the synthetic retinoid isotretinoin [cis-retinoic acid (cis-RA)] in treating minimal residual NB after MAT and established a standard for the use of noncytotoxic differentiation therapy for minimal residual disease.\textsuperscript{164} A recent randomized-controlled trial led by Yu and colleagues\textsuperscript{166} demonstrated that the addition of the anti-GD2 chimeric monoclonal antibody (mAb) in conjunction with cytokines (granulocyte-macrophage colony-stimulation factor and interleukin 2) improved survival, establishing a role for immunotherapy in the standard treatment of HR patients. Additional studies have shown efficacy for different anti-GD2 regimens at diagnosis and recurrence.\textsuperscript{184,185} Future immunotherapy regimens are aimed at determining the importance of cytokines and mAb and examining biomarkers that may predict which patients are most likely to respond favorably to this regimen, which has many side effects, including allergic reactions, fever, hypotension, capillary leak syndrome, and pain (caused by cross-reactivity with GD2 expressed on peripheral nerve cells). Early phase trials are also examining different antibodies and addition of immunomodulators (see “Recurrence” section).

\textbf{LATE EFFECTS}

There are few comprehensive reports of the prevalence of long-term effects in NB survivors, in part because of the poor prognosis for HR NB. Late effects are generally related to chemotherapy/radiation dose intensities, with the highest toxicities in patients who underwent MAT.\textsuperscript{186–190} Recent pharmacogenomic studies have begun to identify germline variants or SNPs that may predict which patients are most susceptible to specific chemotherapy toxicities.\textsuperscript{191} Ototoxicity, renal dysfunction, and endocrine late effects, including hypothyroidism, ovarian dysfunction and infertility, have been detected in most HR patients with NB.\textsuperscript{186} Secondary cancers, most commonly myelodysplastic syndrome and acute myelogenous leukemia, have been reported in 1% to 8% of patients enrolled on trials and small series of NB survivors\textsuperscript{187,192,193} and have been attributed to etoposide exposure, radiation, high-dose MIBG, and

other agents. In addition to hematopoietic malignancies, solid tumors of the thyroid, bone, and kidney have been reported. Patients may also have effects related to tumor location, such as visual impairment caused by orbital metastases and neurologic complications or scoliosis following spinal cord compression.\(^\text{194,195}\)

**RECURRENCE**

Despite recent advances, greater than 50% of patients with HR NB experience tumor recurrence. Although there are no proven curative therapies, some patients achieve prolonged survival even after multiple relapses. In the INRG database, low/IR patients with NB who relapsed had an OS of 65% 5 years after recurrence, whereas for those with metastatic disease, 5-year OS was 8%.\(^\text{196}\) Thus, research into novel therapies is a high priority and has been the subject of several recent reviews.\(^\text{117,196,197}\)

Relapse strategies can be divided into chemotherapies, MIBG/radioisotopes, immunotherapies, and targeted therapies. Current phase I and II trials often involve combinations of these approaches. Cytotoxic chemotherapies commonly used for relapse include topotecan or irinotecan-based regimens\(^\text{170,198–200}\) as well as ifosfamide, carboplatin, etoposide\(^\text{201}\) and often result in transient responses or stable disease but poor long-term survival. Iodide-131- MIBG, which targets high doses of radiation to NB cells, is the most effective single agent for relapsed NB, with response rates greater than 30%.\(^\text{202–205}\) Current MIBG trials will determine the efficacy of concurrent radiosensitizing chemotherapies and feasibility of delivering MIBG followed by MAT to potentially incorporate MIBG into upfront therapy for HR NB.\(^\text{64}\)

Building on the success of anti-GD2 mAbs, novel approaches to enhance mAb efficacy, such as the addition of lenolidomide,\(^\text{206}\) which activates natural killer cells, and active immunization with anti-idiotypic antibodies, are being studied in relapsed patients.\(^\text{207}\) Among the most promising phase I trials are those that use a patient’s own cytotoxic T cells (CTLs) that can be redirected against tumor-associated antigens (eg, GD2, L1CAM). Autologous CTLs engineered to overexpress chimeric antigen receptors are infused and have been shown to persist and demonstrate antitumor activity in patients with NB.\(^\text{208–211}\)

There are several potential targets, and respective inhibitors, for recurrent NB based on preclinical and, in certain cases, phase I trials. A subset of ALK aberrant tumors can be targeted with crizotinib, and trials with second-generation ALK inhibitors and combinations with chemotherapy are underway.\(^\text{37,82,212}\) For patients with MYCNA, preclinical studies suggest that bromodomain and extraterminal domain (BET) inhibitors can induce cell death by interfering with MYCN transcription.\(^\text{91}\) Other drugs that have effects on MYCN stability (aurora kinase A and mTOR inhibitors)\(^\text{213}\) as well as those that target MYC-dependent metabolic changes\(^\text{214}\) are being studied. There is significant interest in drugs, such as histone deacetylase inhibitors, that are less targeted and instead modulate the expression of many genes to induce death, differentiation, and enhance the response to chemotherapies in NB cells.\(^\text{215}\) Other drugs targeting cell cycle (eg, Chk1, Wee-1, CDK4/6), angiogenesis, and differentiation are also under investigation.\(^\text{196}\)

Current trial designs for patients with relapsed NB have incorporated novel approaches, such as pick the winner whereby patients are randomized to receive different novel agents in combination with a common chemotherapy backbone regimens. In addition, many early phase NB trials will incorporate precision medicine by tailoring treatment based on individual patient tumor aberrations. These studies will increasingly depend on genomic and molecular studies of tumors, particularly at the time of relapse, when mutations may be more common.\(^\text{216}\)
FUTURE DIRECTIONS

NB is a heterogeneous tumor for which molecular and genetic determinants affect clinical behavior. Further advances in the understanding of aberrantly expressed genes and pathways will continue to inform and refine risk stratification and treatment and identify novel therapeutic targets. For patients with low risk and IR NB, these genetic factors will help to identify rare patients who still require treatment as we continue to reduce exposures to chemotherapy and surgery for most non-HR patients. In contrast, for HR patients, we need to better predict those at greatest risk of treatment failure or recurrence, either at diagnosis (eg, genetic signatures) or based on their response to treatment (eg, persistent MIBG positive metastases). Furthermore, molecular and genetic studies of tumors at the time of recurrence will be required to specifically identify targets in this chemoresistant population. International collaborations, including INRG databases, are critical for the development of risk stratification and response classifications as well as advances in basic and translational studies, especially for rare populations (eg 4S, OMS). Future studies will move toward more refined risk classifications and treatments based on individual tumor aberrations as well as more attention to survivors to better understand the extent and individual susceptibility to long-term side effects of our treatments.

REFERENCES


