Neuroblastoma, a tumor of the developing peripheral sympathetic nervous system, is the most common and most deadly extracranial solid tumor of childhood. Risk-stratification and risk-adapted therapy play a large role in the modern treatment of neuroblastoma. Recently, through extensive international collaboration, new guidelines for risk stratification have emerged that will aid in international cooperative studies, as well as clarifying therapeutic options for patients. Current therapies for low- and intermediate-risk neuroblastoma have resulted in excellent prognoses for these risk strata, and current efforts are concentrated on chemotherapy reduction. By contrast, much more gradual progress has been made in improving survival for high-risk neuroblastoma patients, despite significant chemotherapy intensification. Current investigations focus on overcoming resistance by elucidating the molecular/genetic causes of neuroblastoma tumorigenesis and progression, with the aim of developing more effective biologically targeted therapies for this disease.

**Keywords:** ALK • INRG • MIBG • MYCN • neuroblastoma • risk stratification

Neuroblastoma is the most common and most deadly extracranial solid tumor of childhood. It accounts for approximately 10% of all childhood cancers, with 10.2 cases per million in children under 15 years old, and for 15% of childhood cancer deaths in the USA [1]. It is a tumor derived from the developing peripheral autonomic nervous system and can have a widely variable presentation and prognosis. Owing to its origin in the neural crest, primary tumors can occur at any place along the peripheral sympathetic chain. Neuroblastoma tumors usually secrete catecholamines and their metabolites, such as vanillylmandelic acid, homovanillic acid and dopamine, which are detectable in the urine or serum of 90% of patients with neuroblastoma. Similarly, tumors also characteristically take up the radiolabeled norepinephrine analog 123I metaiodobenzylguanidine (MIBG), a radiopharmaceutical useful for both diagnosis and staging. Most neuroblastoma tumors occur within the abdomen, with half of these arising from the adrenal glands, but additional sites of origin include the neck, chest and pelvis. Neuroblastoma has been the subject of extensive risk stratification strategies, with the clinical course being dependent on the patient's age at diagnosis, the clinical stage of the tumor, the presence of specific genetic abnormalities and the histologic grade of the tumor.

Overall survival (OS) for patients with neuroblastoma has increased over the last several decades from 45% for patients diagnosed between 1975 and 1979 to 73% for those diagnosed between 2000 and 2006 [2,3]. The clinical course of neuroblastoma can be extremely diverse with a variable clinical presentation as well as prognosis. In general, younger patients (<12–18 months of age) have been shown to have a better prognosis. A subset of tumors in patients less than 1 year of age show spontaneous regression, even with wide dissemination to skin, liver or bone marrow (so-called International Neuroblastoma Staging System [INSS] stage 4S disease) [4]. Interestingly, spontaneous regression is also observed in some patients with localized disease or in children older than 1 year of age [5]. Patients younger than 18 months of age with localized disease and favorable histology
are usually curable with only surgery sometimes combined with modest chemotherapy [3,6–8]. In fact, the majority of patients with intermediate-risk disease also have excellent prognosis – even with recent significant reductions in chemotherapy – with an OS rate of 98% for patients with intermediate-risk tumors with favorable biologic features and 93% for patients with intermediate-risk tumors showing unfavorable features [8]. These findings argue strongly for even more refined risk-stratification strategies for these patients so that intensive chemotherapy, with all of the potential side effects it entails, can be reserved for only those patients who need it.

However, despite this impressive success with low- and intermediate-risk neuroblastoma, patients with high-risk neuroblastoma including older patients (>18 months) with metastases or with unresectable disease with high-risk genetic features (most prominently amplification of the MYCN oncogene) have only a 40% OS. In contrast to intermediate- and low-risk patients, this poor prognosis for high-risk neuroblastoma patients has only improved modestly over the last several decades, despite escalations in the intensity of therapy. For high-risk patients it is clear that intensive research, molecular characterization and specific molecular-targeted therapies may present better opportunities for cure. This article will focus on both the advances and the potential for future progress in risk-stratification and dose-reduction strategies for intermediate- and low-risk neuroblastoma and the current progress towards molecular-targeted and personalized combination therapies for high-risk neuroblastoma.

### Familial neuroblastoma & genetic predisposition

Somatically acquired chromosomal rearrangements and mutations, such as allelic deletions on chromosome 1p or 11q or amplification of the MYCN gene, have been shown to be markers for aggressive, chemotherapy refractory neuroblastoma [9,10]. However, as a disease resulting from the aberrant development of the sympathetic nervous system of very young children, and the occasional occurrence of familial neuroblastoma or association of neuroblastoma with other sympathetic disorders, extensive studies have recently been carried out to look for potential heritable and/or sporadic genetic markers of predisposition. Only 5% of neuroblastoma cases are inherited modestly over the last several decades, despite escalations in the intensity of therapy. For high-risk patients it is clear that intensive research, molecular characterization and specific molecular-targeted therapies may present better opportunities for cure. This article will focus on both the advances and the potential for future progress in risk-stratification and dose-reduction strategies for intermediate- and low-risk neuroblastoma and the current progress towards molecular-targeted and personalized combination therapies for high-risk neuroblastoma.

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### Molecular risk stratification

Numerous genetic risk factors have been implicated and are constantly being proposed for neuroblastoma [28]. DNA content is a significant prognostic indicator with near triploid tumors, predominantly seen in infants, more often localized and with a more favorable prognosis, while near diploid tumors are characterized by high-risk genetic features and more aggressive behavior [29]. Other commonly used genetic markers of increased risk include gains of chromosome arm 17q, loss of 1p or 11q and amplification of the MYCN gene locus [30,31]. Recently, an international task force, the International Neuroblastoma Risk Group (INRG), has published recommendations for additional genetic risk stratification markers to be measured at the time of neuroblastoma diagnostic biopsy [32]. These include the common markers listed above, as well as analysis of the DDX1 gene, NAG gene, mutations or amplifications in the ALK gene, 1q, 2p, 4p, 7q, 9p, 12p and 14q, all of which will be analyzed prospectively for their potential as molecular risk-stratification tools.

One of the most widely accepted and significant molecular risk factors for neuroblastoma is amplification of the MYCN oncogene, which is present in 25–35% of neuroblastoma tumors [10,33]. Historically, tumors with amplified MYCN are almost universally aggressive and chemotherapy resistant, independent of age,
stage or other genetic alterations (although some debate remains regarding the prognostic implications of the rare case of MYCN amplification in stage I disease). In order to overcome this chemotherapy resistance, current strategies use very intensive treatment regimens that have had some success at targeting MYCN amplified disease; however, these strategies also result in significant treatment-related morbidity. Further evidence for the importance of MYCN amplification comes from the TH-MYCN mouse neuroblastoma model in which the human MYCN gene is expressed in the developing neural crest of the mouse, inducing tumors that are histologically and genetically similar to human high-risk disease [22]. Owing to its prominence in high-risk disease, several avenues of research are currently underway to identify means of exploiting therapies that alter Mycn protein levels in neuroblastoma [34].

Many individual gene expression markers, including the TRK family of neurotrophin receptors, have been reported to potentially predict risk in neuroblastoma; however, no individual mRNA expression marker is currently widely accepted as clinically significant enough for incorporation into a clinical risk-stratification schema [35]. Several signaling pathways have been proposed as potentially important in predicting risk, including the PI3K/Akt pathway (which plays an important role in Mycn protein stabilization), the Ret and c-Met pathways, and Src family kinase pathways. Analysis of the activation status of these pathways may yet yield important insight into future molecular risk stratification [34,36].

In the absence of highly statistically significant individual mRNA or protein markers of risk, extensive effort has been devoted to the development of multigene classifiers. The evolution of microarray technology has allowed the development of specific mRNA expression signatures for risk stratification. Numerous studies have been carried out to select reliable genes that will provide accurate risk prediction in individual data sets [37–43]. However, meta-analyses and comparisons between these data sets have shown that, while the technology is maturing and there is some overlap between the several published data sets, it is clear that there is no consensus on the signature useful for clinical risk stratification yet. However, there has been significant overlap in the patterns of gene functionality categories between studies, which may eventually prove clinically useful [44–47]. Other high-throughput technologies for the analysis of large numbers of data points have also shown promise for risk stratification. Recent analysis of patient samples using stem-loop real-time quantitative PCR to simultaneously quantitate the levels of large numbers of noncoding microRNAs has shown promise for a potential microRNA risk-stratification signature [48]. Chip-based array technologies, such as array comparative genomic hybridization (array CGH) and high-throughput sequencing technologies, are continuing to mature and several groups have also proposed whole-genome molecular risk classifications [41,42,49–55]. While there are currently no commercially available assays that have been validated for use in risk-stratification of neuroblastoma, the technology will almost certainly be useful in the future for standardization of the simultaneous measurement of multiple molecular and genomic risk factors identified in the whole genome, mRNA expression and noncoding miRNAs to risk-stratify neuroblastoma patients.

State-of-the-art risk stratification: the INRG classification system

Previous risk-stratification schema for neuroblastoma have included criteria such as the age of the patient at diagnosis, the clinical stage of the tumor, histologic grade of the tumor and the presence or absence of specific genetic features. Despite the need for international collaboration and comparison between cooperative group clinical trials, each of these risk stratification criteria were often differentially applied by each international group, making comparison between trials difficult, if not impossible, as the stratification was often slightly different.

Recently, as a result of extensive international collaboration the INRG has developed a consensus approach to pretreatment risk stratification that uses rigorous statistical methods to determine the most significant prognostic variables and will allow for more detailed comparison between results from different cooperative groups [56]. Using tree-structured analysis, the group used a large cohort of 8800 patients distributed between North America (48%), Europe (47%) and Japan (5%) to divide patients into 16 statistically distinct risk groups based on 13 potential widely accepted prognostic factors. Seven of these prognostic factors remained highly statistically significant and clinically relevant after analysis. The 16 risk groups were then divided into four broad treatment-related categories based on 5-year event-free survival (EFS) rates, including very low risk (<85% 5-year EFS), low risk (75–85% 5-year EFS) intermediate risk (50–75% 5-year EFS), and high risk (<50% 5-year EFS). The most statistically significant prognostic factors were determined to be age at diagnosis and stage, but histologic category, grade of tumor differentiation, MYCN amplification status, presence of 11q aberration and tumor cell ploidy were also significant (Table I). Despite the rigor of this study, it should be noted that many of the gene expression-based predictors were excluded because they were not available for most of the patients. Future studies that include these newer genetic risk factors may provide even better risk stratification.

Age at diagnosis

The INRG committee identified age at diagnosis to be the most important clinical outcome predictor, with patients that were less than 18 months of age having a significantly better OS. The Children’s Oncology Group had previously shown that age at diagnosis is a continuous variable, with gradually worsening OS as the patient’s age increases; however, for the purposes of stratification it was determined by the INRG committee that a cutoff between 570 and 670 days was appropriate and 18 months was determined as the consensus to encourage a convenient and consistent application of the standard [56].

Histology/differentiation status

The prognostic significance of tumor histology and differentiation status in neuroblastoma is well established [57]. The International Neuroblastoma Pathology Classification system (INPC) separates tumors into favorable and unfavorable histologic categories based on Schwannian stromal content, mitosis–karyorrhexis index (MKI), degree of differentiation (differentiating, poorly
differentiated and undifferentiated) and age at diagnosis. This system also divides tumors into four histologic categories by morphologic characteristics, including neuroblastoma (Schwannian stroma-poor), ganglioneuroblastoma intermixed (Schwannian stroma-rich), ganglioneuroma (Schwannian stroma-dominant) and ganglioneuroblastoma nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor). More recently, the INRG committee has implemented the INPC classification system by demonstrating that histologic category, grade of tumor differentiation, MKI and age are all prognostically significant as independent variables [56]. The current INRG classification system uses both histologic category and grade of tumor differentiation in combination with the other five highly significant prognostic markers for risk stratification.

**Staging: INSS versus INRGSS**

Until recently, the most commonly used clinical staging system in both the USA and Europe for neuroblastoma has been the INSS, which was developed by an expert panel in 1988 and revised in 1993. This system is based primarily on surgical outcomes, which allow for significant variability in staging for patients with neuroblastoma depending on degree of surgical resection or local variations in surgical practice. In order to address these limitations, the International Neuroblastoma Risk Group Staging System (INRGSS) was developed in 2009 [58]. This new system uses specific image-defined risk factors (IDRFs) that carefully define the practicality of safe, total surgical tumor excision using objective imaging criteria rather than postoperative analysis. Determination of IDRFs requires standardization of required imaging studies, including MRIs or CT scans of primary and known metastatic sites, iodine-123 I MIBG scintigraphy for the diagnosis of metastatic disease and (in the absence of MIBG-positive primary disease) Technetium-99 bone scintigraphy [59]. It should be noted that INRGSS criteria will probably not change prognostic impact much compared with INSS staging [60]; however, when using INRGSS criteria, in combination with centralized radiologic review, staging can be more easily standardized and different therapeutic strategies can be directly compared between cooperative groups (Table 2).

**Modern therapeutic strategies in the age of intensive risk stratification**

**Low- & intermediate-risk disease**

Current Children’s Oncology Group (COG) trials use overall risk classification using age, INSS stage, MYCN amplification, INPC histopathology and DNA index to divide neuroblastoma into treatment groups with escalating intensity of therapy. Low risk, localized disease in younger patients has an excellent prognosis and is often managed with surgery alone, resulting in >95% 4-year OS. As mentioned previously, recently published data from the COG in patients with intermediate-risk disease (infants whose tumors did not show MYCN amplification, and who had INSS stage 3 or 4 disease or INSS stage 4S disease with unfavorable histology or DNA index; older children with stage 3 MYCN-nonamplified tumors with favorable pathology) showed a 96% 3-year OS with surgery and a short 3–6 month course of outpatient chemotherapy [8].

The current COG Phase III trial for intermediate-risk neuroblastoma uses previously defined pretreatment clinical and biological risk factors, now including 1p or 11q deletion, to stratify patients into treatment groups for their initial chemotherapy regimens. In light of the extremely promising results of the previous intermediate-risk trial, further personalization of therapy is being attempted in these patients by incorporating the additional

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**Table 1. International Risk Group Classification System.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Age (months)</th>
<th>Histologic category</th>
<th>Grade of differentiation</th>
<th>MYCN Amp</th>
<th>11q aberration</th>
<th>Ploidy</th>
<th>Pretreatment risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1/2</td>
<td></td>
<td>GN maturing; GNB intermixed</td>
<td>No</td>
<td>Yes</td>
<td>A: Very low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td></td>
<td>Any, except GN maturing or GNB intermixed</td>
<td>No</td>
<td>Yes</td>
<td>B: Very low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>&lt;18</td>
<td>Any, except GN maturing or GNB intermixed GNB nodular; neuroblastoma</td>
<td>No</td>
<td>Yes</td>
<td>D: Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥18</td>
<td>Poorly differentiated or undifferentiated</td>
<td>No</td>
<td>Yes</td>
<td>G: Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>&lt;18</td>
<td>No</td>
<td>No</td>
<td>Hyperdiploid</td>
<td>F: Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;12</td>
<td>No</td>
<td>No</td>
<td>Diploid</td>
<td>I: Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 to &lt;18</td>
<td>Yes</td>
<td>No</td>
<td>Diploid</td>
<td>J: Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;18</td>
<td>No</td>
<td>Yes</td>
<td>O: High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥18</td>
<td>Yes</td>
<td>Yes</td>
<td>P: High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>&lt;18</td>
<td>No</td>
<td>No</td>
<td>C: Very low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>Q: High</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Amp: Amplified; GN: Ganglioneuroma; GNB: Ganglioneuroblastoma. Reproduced with permission from [56].
genetic factors, chemotherapy and surgical response criteria to further define the risk of relapse or recurrence after initial induction therapy, and to either intensify or reduce therapy accordingly. In this way, current trials will continue to reduce the morbidity and mortality of treatment for low- and intermediate-risk neuroblastoma. In the future, through refinements of risk stratification, such as the INRG classification system, and largely through the prospective study of important molecular and genetic markers for high-risk disease and chemotherapy resistance, the hope is to predict chemotherapy response in intermediate-risk disease before treatment, and therefore avoid acute or late toxicity.

Risk stratification & conventional therapy for high-risk neuroblastoma

While future trials will almost certainly use the new INRG risk classification, current COG trials classify high-risk disease as those patients with INSS stage 4 disease who are older than 18 months at diagnosis; INSS stage 2, 3, 4 or 4S disease with MYCN amplification; and INSS stage III disease with age >18 months and unfavorable histology. Current conventional therapy for neuroblastoma can be divided into the induction of remission, consolidation and maintenance phases. Currently, most high-risk induction regimens include cycles of cisplatin/etoposide alternating with vincristine, doxorubicin and cyclophosphamide, with the more recent addition of topotecan [61,62]. As mentioned previously, further efforts at chemotherapy intensification during the induction phase with conventional agents, such as alkylating agents, anthracyclines, topoisomerase inhibitors and platinum derivatives, met with some initial success, but have resulted in little improvement of EFS or OS [63]. Efforts are also underway to determine, at a molecular level, the extent of trace minimal residual disease in the peripheral blood after induction of gross remission as a means of further risk and therapy stratification [64].

Recent progress has been attained through intensification of the consolidation phase of high-risk treatment by using myeloablative therapy with hematopoietic stem cell rescue. This intensification did significantly increase OS when compared with previous trials [65–68]. The current COG high-risk protocol attempts to further intensify this consolidation phase through the use of two sequential, tandem myeloablative treatments, each followed by stem cell rescue [69].

The improvement in OS by the introduction of myeloablative consolidation was further enhanced by the addition of a maintenance phase of treatment using isotretinoin (13-cis-retinoic acid), which is a differentiating agent used for the treatment of minimal residual disease to prevent relapse (which occurs in 40% of high-risk neuroblastoma cases) [68]. Trials are also underway to attempt to improve this minimal residual disease therapy during maintenance through the use of an alternate retinoid, fenretinide, which is also thought to induce apoptosis of residual neuroblastoma cells via the ceramide pathway [70–72]. Despite these significant advances in delivering dose-intensive therapy, long-term survival for these high-risk patients remains poor and it is clear that further dose intensification will probably result in extensive chemotherapy-related morbidity. Ideally, our current and extensive knowledge of neuroblastoma biology may be refined, extended and used to develop novel targeted therapies. In fact, two novel targeted therapies have recently emerged for the upfront treatment of high-risk neuroblastoma.

‘Proven’ targeted therapies for high-risk neuroblastoma

Relapse occurs in approximately 40% of all high-risk neuroblastoma cases and recent progress has been made towards the elimination of minimal residual disease with postconsolidation antibody-targeted maintenance therapy. Added to existing isotretinoin maintenance, immunotherapy with the monoclonal antibody ch14.18, which is directed against the tumor-associated disialoganglioside GD2 (commonly expressed on neuroblastoma tumors), in combination with immunomodulatory cytokines, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-2, showed significant improvement in 2-year EFS and OS after high-dose myeloablative consolidation therapy [73]. While it does have significant immediate toxicity and continues to be refined, this immunotherapeutic cocktail in addition to the standard therapy with isotretinoin has been incorporated into the established maintenance for high-risk neuroblastoma.

Metaiodobenzylguanidine is a norepinephrine analogue that is concentrated in neural crest tissues, where it is taken up via the norepinephrine transporter. MIBG is taken up by 90–95% of neuroblastoma tumors, and when it is labeled with a radioisotope of iodine, it may be used very effectively for both treatment and imaging of neuroblastoma [74]. Neuroblastoma is known to be radiosensitive and external-beam radiation is commonly used, often in combination with surgery, for control of local and focal metastatic disease [75]. The efficient targeting of radioisotopes to the tumors by MIBG has the advantage of specifically delivering a high-dose of radiation to both primary, metastatic and micro-metastatic disease over an extended period of time, while...
largely sparing normal tissues. When labeled with $^{131}$I, MIBG treatment results in a 30–40% response rate in highly treatment-refractory cohorts of patients with relapsed neuroblastoma [76]. New COG clinical trials will incorporate the use of $^{131}$I MIBG as upfront consolidation therapy in combination with myeloablative chemotherapy and hematopoietic stem cell rescue, an approach that has shown promise in Phase I clinical trials of patients with refractory disease [77].

**Novel targeted therapies for high-risk neuroblastoma**

As mentioned previously, there are numerous known molecular risk factors for high-risk neuroblastoma that have been discovered through genetic mutational analyses, mRNA expression analysis and signaling pathway analysis that provide potential targets for therapeutic intervention (outlined in Figure 1). In addition to individual laboratory efforts, collaborative groups, such as the TARGET program (which, through genomic profiling and massive sequencing efforts, seeks to identify target pathways and molecules that may be good therapeutic targets [201]) and the Pediatric Preclinical Testing Program (which uses mouse xenograft models of pediatric tumors to test drugs that are already in development for adult tumors in an effort to find new therapies [202]) are providing technologically current and high-throughput techniques to identify new drugs that are active against high-risk neuroblastoma. In addition, the New Advances in Neuroblastoma Therapy (NANT) cooperative group has provided a more rapid and facilitated means of testing new drugs in early phase clinical trials [203].

The recent discovery of the prevalence of both germline and somatic ALK mutations in neuroblastoma certainly argues for the importance of this molecule in tumorigenesis and, potentially, progression. Inhibitors of the ALK receptor tyrosine kinase are currently in clinical development for the treatment of anaplastic lymphoma and promising preclinical data has led to Phase I and II clinical trials of ALK-targeted therapies in neuroblastoma in the COG [16,204,205]. Recent results suggest that only certain mutations are likely to respond to such inhibitors, so genetic testing of tumors at diagnosis and relapse is critical [16,78].

Another possible avenue of investigation is the synthesis of common pathway abnormalities that are known to be important in high-risk neuroblastoma to predict which interventions will have the highest likelihood of success. For example, the critical importance of MYCN amplification as a prominent molecular risk factor implies that upstream pathways that alter Mycn expression may be good targets for intervention. Aurora kinase A is a known cell-cycle regulator that has been shown to also be a key modulator of Mycn protein stability [79]. Interestingly, an inhibitor of Aurora kinase A, MLN8237, has also been shown to have promising activity against neuroblastoma xenografts in testing by the Pediatric Preclinical Testing Program and Phase I testing in human patients is currently underway [80]. Other upstream regulators of Mycn protein stability include members of the PI3K/AKT pathway, and preclinical trials of inhibitors of this pathway show some promise in neuroblastoma; however, more definitive clinical trials have yet to begin [34]. Interestingly, recent data have shown a correlation between specific oncogenic-activating mutations in the ALK gene and MYCN-amplified disease perhaps providing a link between these two molecules, which are both clearly critical in neuroblastosoma tumorigenesis, and potentially providing further opportunities for therapeutic intervention through combination therapies [81].

Additional preclinical data is emerging for a number of other novel targeted molecular therapies in neuroblastoma. Targeted alteration of the tumor microenvironment, using drugs such as the angiogenesis inhibitor bevacizumab, alone or in combination with conventional chemotherapeutic agents, is a promising strategy currently being exploited, owing to the overexpression of VEGF receptors in high-risk neuroblastoma [82,83].

Another novel approach is the alteration of the epigenetic structure of the genome through the use of histone deacetylase inhibitors. These drugs sensitize neuroblastoma cells to multimodality therapy, such as chemotherapy, external beam radiation or MIBG radiotherapy, and clinical trials are currently underway to test their effectiveness in patients [84–86]. The success of anti-GD2 and IL-2 immunotherapy as a treatment of minimal residual disease has raised the possibility of other immunologically directed therapies. Specifically, the use of unrelated umbilical cord blood transplant after myeloablative consolidation therapy allows the addition of natural killer cellular immunotherapy to attack residual neuroblastoma cells [87]. There have also been several approaches attempted in mouse models at making an antineuroblastoma vaccine, including a vaccine against tyrosine hydroxylase (which is commonly expressed in neural crest tissue), against the GD2 cell surface marker for neuroblastoma, against fusion proteins with cytokines, such as IL-2, TNF-α and IL-21, and against a survivin minigene (consisting of immunogenic peptide sequences of the antiapoptotic gene, survivin) [88–91]. Figure 2 illustrates some of the most promising current targeted therapeutic approaches being taken. Clearly, as our knowledge of the molecular basis of neuroblastoma biology broadens so will our opportunities for targeted intervention and rational drug design for the much needed treatment of high-risk disease.

**Expert commentary**

Extensive progress has been made towards more accurate risk-stratification and personalized therapy in neuroblastoma. The INRG committee recommendations and the INRGSS provide a foundation for further international collaboration and for refinement of existing risk-stratification criteria. Risk- and response-based stratification in neuroblastoma has resulted in excellent clinical outcomes for low- and intermediate-risk disease, and is allowing further morbidity sparing chemotherapy dose reduction for patients through ongoing clinical trials. Regarding high-risk disease, the benefit of additional intensification of cytotoxic and myeloablative chemotherapy appears to have been reached. However, novel and specific neuroblastoma-targeted $^{131}$I MIBG-consolidation therapy and disease-targeted immunotherapy are now being incorporated into frontline treatments for high-risk neuroblastoma in the hopes of continuing to improve outcomes.
Figure 1. Key molecules for targeted pharmacotherapy in neuroblastoma. The PI3K/AKT pathway has been implicated as being critical to multiple neuroblastoma tumorigenesis and chemotherapy-resistance pathways. The pathway is shown here with known drugs in clinical development highlighted in boxes; each drug target is noted in parentheses. Furthest upstream in the pathway are several RTKs, including ALK [16], inhibitors that target multiple RTKs, such as sunitinib, sorafenib, dasatinib and gefitinib [92–95], inhibitors that target Trks, such as lestaurtinib [96], and monoclonal antibody IMC-A12, which targets the IGF-1R [97]. Downstream pathway targeted inhibitors include those against PI3K [98,99], AKT [100], mTOR complex inhibitors (for a review, see [101]) and Aurora Kinase A inhibitors [79,80]. Other classes of targeted inhibitors include those against histone deacetylase [102] and against the canonical p53 apoptosis pathway [103].

EGFR: EGF receptor; HDAC: Histone deacetylase; RTK: Receptor tyrosine kinase; VEGF: VEGF receptor.
Several biologically targeted agents have been used against neuroblastoma, including the targeted radiotherapeutic agent $^{131}$I MIBG, which is taken up by the norepinephrine transporter and induces radiation damage specifically to neuroblastoma tumors [76,77]. Immunotherapy acts either induction of immunogenicity by a vaccine or through the direct targeting of tumor cells by monoclonal antibodies, and has also been tried with some significant success using antibodies directed against GD2, among other molecules [73,87–91,104]. These molecules are thought to function through modulation of antibody-dependent cellular cytotoxicity where the host’s own immune response mediates the antitumor effect. Inhibitors of angiogenesis, such as bevacizumab and zoledronic acid, are also being tested to prevent neovascularization of neuroblastomas [82,83]. Retinoids in clinical trials include isotretinoin, which targets the RA receptor, and fenretinide, which targets both the RA receptor and ceramide-induced apoptosis in neuroblastoma cells [70–72].

MIBG: Metaiodobenzilguanidine; RA: Retinoic acid.
and reduce toxicity. With the help of massive efforts by both individual researchers and collaborative groups using high-throughput methods for high-resolution molecular characterization of neuroblastoma tumors, a number of potential targets for therapy have been elucidated and are currently being exploited in preclinical and early-phase clinical trials.

Five-year view
The results of the most recent intermediate-risk and high-risk neuroblastoma trials will further emphasize the importance of risk stratification in this disease. It is likely that the next set of intermediate-risk clinical trials emerging over the next several years will involve even further risk stratification, response-based stratification and reduction or even elimination of chemotherapy in some subgroups with improved survival. For high-risk disease, the initial results of pilot studies of augmented upfront therapy consolidation with 131I MIBG and augmented maintenance immunotherapy will be available within the next 3–4 years and will likely be expanded to a full Phase III randomized clinical trial. Further refinement of the molecular diagnostic techniques, such as chip-based assays, and the accurate measurement of minimal residual disease in high-risk neuroblastoma will allow additional response and biology-based risk stratification. Within the next several years through the high-throughput sequencing and analysis of tumor samples from several collaborative groups the vast majority of mutations necessary for neuroblastoma oncogenesis and tumor progression will be elucidated. Once these key mutations are identified, and the genes that they encode are further characterized, they will present prime targets for rational drug design and personalized targeted therapies. However, in light of the complexity and heterogeneity of neuroblastoma biology, it is likely that combination of these targeted therapies with one another and with conventional multimodality therapy will be necessary in order to make large improvements in EFS and OS for high-risk neuroblastoma.

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Key issues
- Neuroblastoma is the most common and deadly extracranial solid tumor of childhood, accounting for a disproportionate number of childhood cancer deaths.
- The understanding of clinical and molecular risk stratification and risk-directed therapy has been refined extensively over the past several decades. Extensive research efforts are currently underway to add to our molecular understanding of this disease.
- The International Neuroblastoma Risk Group has recently developed a rigorously defined set of clinical and molecular risk-stratification criteria, which use age at diagnosis, International Neuroblastoma Risk Group Staging System stage at diagnosis (as defined by specific presurgical image-defined risk factors), histologic classification of tumor grade and degree of differentiation, as well as the molecular risk factors of DNA ploidy, MYCN amplification and 11q aberration to stratify patients into 16 individual risk categories, further dividing these categories into high-risk, intermediate-risk, low-risk and very low-risk treatment groups.
- The treatment strategies for low- and intermediate-risk neuroblastoma have been extremely successful and the current clinical trials are now focused on further risk stratification and dose reduction to spare patients from treatment-related morbidity and mortality.
- High-risk neuroblastoma still presents a clinical challenge and, despite some improvement in survival with extensive chemotherapy treatment intensification, including the addition of myeloablative therapy with hematopoietic stem cell rescue, survival is still relatively poor.
- Future treatment strategies for high-risk disease will focus on targeted and disease-specific therapies. Among these targeted therapies, augmented consolidation with targeted radiotherapy using 131I MIBG and immunotherapy with neuroblastoma-specific anti-GD2 monoclonal antibody and cytokine therapy in maintenance have already shown great promise for improvement in survival of high-risk neuroblastoma.
- Targeted molecular therapy with rationally designed drugs, such as inhibitors of the anaplastic lymphoma kinase receptor tyrosine kinase, histone deacetylase and Aurora kinase A, among others, are also in preclinical and early phase trials for neuroblastoma and show great promise for the improvement of treatment for high-risk disease.

References
Papers of special note have been highlighted as:
• of interest
** of considerable interest
Showed ALK to be the major genetic predisposition factor in neuroblastoma.


**Showed ALK to be the major genetic predisposition factor in neuroblastoma.


**Showed ALK to be the major genetic predisposition factor in neuroblastoma.


**Showed ALK to be the major genetic predisposition factor in neuroblastoma.


**First genetically engineered mouse model of neuroblastoma.


Progress towards personalized therapeutics: biologic- & risk-directed therapy for neuroblastoma


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• Key international consensus classification system for neuroblastoma.


• Key international consensus classification of neuroblastoma tumors.


• Key imaging evaluation system for neuroblastoma classification.


• Critical intensity escalating treatment strategy for high-risk disease.


• Key outcome data for high-risk patients.
Critical recent article showing a new target for novel therapies.


Progress towards personalized therapeutics: biologic- & risk-directed therapy for neuroblastoma


**Landmark finding that immunotherapy significantly prolongs survival in neuroblastoma.**

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