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Progress in treatment and risk stratification of neuroblastoma: Impact on future clinical and basic research.

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Abstract: Close international collaboration between pediatric oncologists has led to marked improvements in the cure of patients, seen as a long-term overall survival rate of about 80%. Despite this progress, neuroblastoma remains a challenging disease for both clinicians and researchers. Major clinical problems include lack of acceptable cure rates in high-risk neuroblastoma and potential overtreatment of subsets of patients at low and intermediate risk of the disease. Many years of intensive international cooperation have recently led to a promising joint effort to further improve risk classification for treatment stratification, the new International Neuroblastoma Risk Group Classification System. This approach will facilitate comparison of the results of clinical trials performed by different international collaborative groups. This, in turn, should accelerate refinement of risk stratification and thereby aid selection of appropriate therapies for individual patients. To be able to identify new therapeutic modalities, it will be necessary to elucidate the pathogenesis of the different subtypes of neuroblastoma. Basic and translational research have provided new tools for molecular characterization of blood and tumor samples including high-throughput technologies for analysis of DNA, mRNAs, microRNAs and other non-coding RNAs, as well as proteins and epigenetic markers. Most of these studies are array-based in design. In neuroblastoma research they aim to refine risk group stratification through incorporation of molecular tumor fingerprints and also to enable personalized treatment modalities by describing the underlying pathogenesis and aberrant signaling pathways in individual tumors. To make optimal use of these new technologies for the benefit of the patient, it is crucial to have a systematic and detailed documentation of both clinical and molecular data from diagnosis through treatment to follow-up. Close collaboration between clinicians and basic scientists will provide access to combined clinical and molecular data sets and will create more efficient steps in response to the remaining treatment challenges. This review describes the current efforts and trends in neuroblastoma research from a clinical perspective in order to highlight the urgent clinical problems we must address together with basic researchers.

Lund, Sweden, 2011-04-18

LETTER TO THE EDITOR

We are honored and pleased as invited authors to submit the manuscript:

Progress in treatment and risk stratification of neuroblastoma: - impact on future clinical and basic research” by Ingrid Øra and Angelika Eggert to Seminars in Cancer Biology.

The manuscript is part of a series of invited manuscripts involving research of neuroblastoma, a childhood tumor with a complex heterogeneity both in presentation and clinical course. This review describe the disease and current trends in research from a clinical perspective with the aim to highlight the major challenges we need to address together with basic and preclinical neuroblastoma researchers.

Sincerely

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Progress in treatment and risk stratification of neuroblastoma: - impact on future clinical and basic research

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Keywords: neuroblastoma; risk group; treatment stratification; clinical trial; International Neuroblastoma Risk Group Classification; collaboration

Abstract

Close international collaboration between pediatric oncologists has led to marked improvements in the cure of patients, seen as a long-term overall survival rate of about 80%. Despite this progress, neuroblastoma remains a challenging disease for both clinicians and researchers. Major clinical problems include lack of acceptable cure rates in high-risk neuroblastoma and potential overtreatment of subsets of patients at low and intermediate risk of the disease. Many years of intensive international cooperation have recently led to a promising joint effort to further improve risk classification for treatment stratification, the new International Neuroblastoma Risk Group Classification System. This approach will facilitate comparison of the results of clinical trials performed by different international collaborative groups. This, in turn, should accelerate refinement of risk stratification and thereby aid selection of appropriate therapies for individual patients. To be able to identify new therapeutic modalities, it will be necessary to elucidate the pathogenesis of the different subtypes of neuroblastoma. Basic and translational research have provided new tools for molecular characterization of blood and tumor samples including high-throughput technologies for analysis of DNA, mRNAs, microRNAs and other non-coding RNAs, as well as proteins and epigenetic markers. Most of these studies are array-based in design. In neuroblastoma research they aim to refine risk group stratification through incorporation of molecular tumor fingerprints and also to enable personalized treatment modalities by describing the underlying pathogenesis and aberrant signaling pathways in individual tumors. To make optimal use of these new technologies for the benefit of the patient, it is crucial to have a systematic and detailed documentation of both clinical and molecular data from diagnosis through treatment to follow-up. Close collaboration between clinicians and basic scientists will provide access to combined clinical and molecular data sets and will create more efficient steps in response to the remaining treatment challenges. This review describes the current efforts and trends in neuroblastoma research from a clinical perspective in order to highlight the urgent clinical problems we must address together with basic researchers.

Introduction

Treatment of children with neuroblastoma is slowly but steadily improving, which is reflected by somewhat better survival rates in patients with high-risk disease and by successful treatment reduction strategies based on appropriate risk stratification in cases of low and intermediate risk disease [1-6]. Many years of intensive international collaboration have

1 recently led to the International Neuroblastoma Risk Group (INRG) Classification System, a
2 promising effort that can improve treatment stratification [7]. This system will facilitate
3 comparison of the results of clinical trials performed by collaborative groups in different parts
4 of the world, and it will probably also accelerated refinement of risk stratification for selection
5 of appropriate therapies for individual patients.

6 There are two major challenges in clinical neuroblastoma research: the absence of
7 acceptable progress in cure rates for high-risk neuroblastoma patients [8, 9] and the potential
8 overtreatment of other patients [5, 10, 11]. Furthermore, there is growing evidence that the
9 patients we cure today are at considerable risk of late complications of the treatment they
10 have received [12-15]. There is a huge need for additional and innovative treatment
11 modalities, in particular for children with high-risk neuroblastoma. Despite this situation,
12 encouraging novel therapeutic developments have been made during the past years, which
13 suggests that we are on the right track.

14 A large number of dedicated clinical and preclinical researchers work daily to overcome the
15 existing obstacles in neuroblastoma treatment. Basic and translational research have
16 provided new tools for molecular characterization of tumor samples, which include high-
17 throughput technologies as comparative genomic hybridization (CGH), single nucleotide
18 polymorphism (SNP), or next generation sequencing (NGS), mRNAs, microRNAs and other
19 non-coding RNAs, as well as proteins and epigenetic markers. Most of these investigations
20 are array-based and they aim to refine risk stratification and also to describe the underlying
21 tumor pathogenesis for the identification of new targets for treatment.

22 To achieve the greatest benefit for patients when using the plethora of new molecular
23 technologies in tumor profiling, it is essential to obtain a detailed surveillance and
24 documentation of clinical and molecular data collected from diagnosis through treatment and
25 follow-up. Therefore, for researchers applying the powerful array-based technologies to
26 neuroblastoma samples, it is of the utmost importance to work closely together with clinicians
27 and to have access to detailed clinical data so that the molecular data obtained can be
28 correctly interpreted within the context of the many heterogeneous aspects of
29 neuroblastoma. The close national and international cooperation of pediatric oncologists has
30 led to the great advances in treatment of children with cancer and has resulted in the present
31 overall long-term survival rate of nearly 80% [16-18]. Clearly, this indicates that intensifying
32 the collaboration with basic scientists will result in more efficient steps towards our goals. In
33 this review we describe neuroblastoma and the current efforts and trends in neuroblastoma
34 research from a clinical perspective with the aim of highlighting the urgent clinical problems
35 we must address together with preclinical researchers.

40 **History of Neuroblastoma**

41 A century ago, in 1910, the pathologist J.H. Wright introduced the name neuroblastoma for a
42 childhood tumor of neuronal origin [19]. Wright collected cases previously diagnosed as
43 sarcomas, in which he recognized neural fibrils and bundles of what resembled immature
44 cells in the fetal adrenal medulla. In a recent publication giving a historical perspective on the
45 first reported cases of neuroblastoma, the early attempts to treat this disease after World
46 War I are described, which were initially limited to pediatric surgery [20]. Surgery was indeed
47 successful in cases with localized disease in a later review of 217 cases [21]. In cases with
48 larger tumors or where complete surgery could not be achieved, the introduction of
49 orthovoltage X-ray therapy rescued a subset of the patients [22]. Decades before the
50 introduction of chemotherapy, it was observed that the chance of survival was better in
51 infants than in older children with more advanced disease. Early on, clinicians recognized
52 that infants with metastatic spread confined to the skin and liver could undergo spontaneous
53 remission without treatment intervention [23]. The first reports of extended survival after
54 chemotherapy in children with neuroblastoma were published in 1960s, although most of the
55 patients relapsed [24, 25]. Some years later, Dr. Audrey Evans developed the first staging
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1 system that proved to be of great importance for the advancement and harmonization of
2 neuroblastoma treatment [26].

3 **Epidemiology**

4 Neuroblastoma affects mainly infants and young children and accounts for 7–10% of all
5 pediatric malignancies. The age distribution is characterized by a peak incidence in the first
6 year of life, followed by a rapid decline in subsequent years. The median age of diagnosis is
7 approximately 20 months, and 90% of cases are diagnosed by the age of 6 years. In
8 Western countries, the annual incidence of neuroblastoma is estimated to be 10.9 per million
9 children below 15 years of age and it occurs in 1 of 7 000 live births [27]. The overall
10 influence of known environmental agents on the etiology of neuroblastic tumors is very low
11 and the consistent incidence rates of neuroblastoma in children support the hypothesis of a
12 major role of genetic factors [28, 29].

13 The tumor is thought to arise from neural crest-derived cells that form the developing
14 sympathetic nervous system in the embryo and fetus and are often described as being
15 arrested at an early stage of differentiation. After migrating from the neural crest, the
16 pluripotent sympathogonia form the sympathetic ganglia, the chromaffin cells of the adrenal
17 medulla and the paraganglia, which represent the typical locations of neuroblastomas [30].

18 Neuroblastomas belong to the “small blue round cell” neoplasms of childhood and to the
19 group of peripheral neuroblastic tumors (pNTs) [31], which includes neuroblastomas (NB),
20 ganglioneuroblastomas (GNB) and the benign ganglioneuromas (GN). These tumor types
21 reflect different degrees of maturation, ranging from undifferentiated cells with large dense
22 nuclei and scant cytoplasm to poorly differentiated and differentiating cells, and finally to
23 ganglion cells with inclusion of neurophils and Schwann cells with increased maturation.

24 The International Neuroblastoma Pathology Classification, INCP, developed by Shimada *et al.*
25 [32, 33] considers the impact of several histopathological features and the mitosis-
26 karyorrhexis index of the tumor, together with the age of the patient at diagnosis. The INCP
27 assigns pNTs to one of four basic morphological categories, which are designed as follows:
28 NB (Schwannian stroma-poor), GNB intermixed (Schwannian stroma-rich), GNB nodular
29 (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor) and GN
30 (Schwannian stroma-dominant) [34]. The neuroblastoma category comprises three subtypes
31 denoted (1) undifferentiated, (2) poorly differentiated and (3) differentiating. The INPC
32 system has been further refined and widely adapted to identify favorable and unfavorable
33 tumor subtypes for treatment stratification. The morphological features described the INPC
34 are significant correlated with the biological properties of the pNTS, such as *MYCN*
35 amplification or *TrkA* expression.

36 As patient age is a covariate in the INCP, and pathologists experienced in applying the
37 Shimada classification system are not always available at small centers, Cohn *et al.* [7]
38 recently proposed the International Neuroblastoma Risk Group (INRG) Classification
39 System. The INRG classification incorporates only the basic histopathological categories
40 (favorable GN-maturing or GNB-intermixed versus unfavorable GNB-nodular or NB) and
41 tumor cell differentiation (differentiating, poorly differentiated, or undifferentiated) to achieve a
42 global treatment stratification system (see below)

43 **Neuroblastoma predisposition and genetics**

44 *Familial neuroblastoma*

45 A family history of neuroblastoma is observed in approximately 1% of patients.
46 Neuroblastoma pedigrees usually show an autosomal dominant pattern of inheritance with
47 incomplete penetrance. At least two neural crest-derived developmental disorders are
48 associated with an increased risk of neuroblastoma: Hirschsprung’s disease, which is
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1 characterized by absence of ganglion cells in the distal colon, resulting in functional
2 obstruction; Ondine's curse, which involves a failure of the autonomic control of ventilation
3 during sleep. These two diseases are frequently interrelated, and most cases are linked to
4 mutation of the *PHOX2B* gene, which is associated with differentiation of the sympathetic
5 nervous system and synthesis of catecholamine [35, 36]. Although the involvement of
6 *PHOX2B* in familial cases of neuroblastoma is compelling, the contribution of this gene to the
7 development of sporadic neuroblastoma is much less obvious because somatic mutations
8 are extremely rare [37, 38].

9 More recently, the anaplastic lymphoma kinase (*ALK*) gene was also identified as
10 predisposing to neuroblastoma in studies that demonstrated germline mutations in *ALK* in
11 neuroblastoma pedigrees [39, 40]. The *ALK* gene encodes a transmembrane receptor
12 tyrosine kinase that is known to be preferentially expressed in the central and peripheral
13 nervous systems, but the functions of this protein is poorly understood [41]. To date, three
14 types of *ALK* germline mutations have been described in neuroblastoma families, and the
15 most frequent occurring mutation is designated R1275Q. Although detailed clinical
16 information is still lacking for several families, it seems likely that the penetrance of these
17 mutations is incomplete, and neuroblastic tumors of varying aggressiveness can be observed
18 in carriers of an *ALK* mutation.

21 *Sporadic neuroblastoma*

22 Although neuroblastoma can occur in familial contexts, most cases arise sporadically. The
23 development of high-resolution array CGH has allowed comprehensive examination of
24 whole-genome patterns of aberrations in neuroblastoma tumors and cell lines [42-47].

25 The oncogene *MYCN* on chromosome 2p24 is amplified in about 20% of all tumors and is
26 highly associated with poor outcome despite age [48-50]. The adverse prognostic effect of
27 *MYCN* amplification on outcome has been confirmed in many studies, and *MYCN* status is
28 routinely used in clinical practice in all of the current collaborative trials to assign therapeutic
29 intensity.

30 In addition to *MYCN* amplification, several other cytogenetic alterations have been described
31 in primary neuroblastomas, the majority of which represent allelic losses of chromosomal
32 material or whole chromosome gains. Segmental copy number alterations occur often, and
33 these mainly involve chromosome deletions (1p, 3p, and 11q) and gains (1q, 2p, and 17q)
34 and are usually associated with a poor outcome [51-55]. For many of these aberrations, the
35 prognostic value in retrospective studies tends to disappear in multivariate analysis, although
36 it is plausible that further studies will reveal tumor subgroups with specific phenotype and
37 clinical behavior [56, 57].

38 Loss of 1p36 has been observed in 23–35% of neuroblastoma tumors, and has been shown
39 to be significantly associated with prognostic markers of aggressive disease [58-60].
40 Therefore, it seems likely that the genomic region of 1p36 contains one or more
41 neuroblastoma tumor-suppressor genes, which to date have not been identified. Deletion of
42 1p36 has been found to predict survival in multivariate analyses, but the independent
43 prognostic value is still controversial. However, some studies have shown increased relapse
44 rates in cases involving low and intermediate neuroblastomas with 1p36 deletion, although
45 these relapsed patients could be rescued with intensified treatment approaches [7]. The
46 1p36 deletion is currently being used to stratify treatment in an ongoing neuroblastoma trial
47 in Germany [61].

48 More recently, the effect of loss chromosome 11q on the outcome of neuroblastoma patients
49 has been determined. Deletion of 11q in regions in 11q23 has been detected in 26–44% of
50 cases in large patient cohorts. Interestingly, although loss of 11q is associated with features
51 that are unfavorable in neuroblastoma, it is inversely correlated with *MYCN* amplification
52 Thus, the occurrence of 11q deletions and the presence of *MYCN* amplification appears to
53 represent two molecularly distinct subgroups of aggressive neuroblastoma. In multivariate
54 analyses of relevant prognostic variables, allelic loss of 11q was found to be an independent
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1 marker of decreased event-free survival in entire cohorts as well as in subgroups of low- and
2 intermediate-risk cases [50, 62-64]. Thus, 11q alterations represent a prognostic marker for
3 improved risk- stratification of neuroblastoma patients.

4 An unbalanced gain of chromosome 17q occurs in > 50% of neuroblastomas, and gains of
5 whole chromosome 17 is seen in 40% of the hyperdiploid cases [65]. Although, the 17q gain
6 has been observed to have prognostic value in subgroups it is not strong or independent
7 enough to be included in clinical trials.

8 Somatic and activating mutations of the *ALK* gene were recently identified in approximately
9 8% of neuroblastoma tumors, and this constitutes a breakthrough in understanding of the
10 pathogenesis of this disease [39, 40, 66, 67]. Interestingly, the spectra of somatic and germ-
11 line *ALK* mutations differ. The existence of a link between such aberration and tumor biology
12 has not yet been fully determined, since the studies published so far have revealed no
13 consistent correlations between *ALK* mutations and aggressive neuroblastoma subtypes.
14 Analysis of larger neuroblastoma series will provide further information about the precise
15 relationship between the tumor phenotype and alterations in *ALK* mutations and/or genomic
16 regions.
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18 Early investigations demonstrated prognostic implication of ploidy (or DNA index) in
19 neuroblastoma and this has been used for treatment stratification in several clinical trials in
20 Germany and the United States. Studies have shown that in contrast to near-triploid tumors,
21 near-diploid lesions constitute a risk factor for patients with metastatic disease between 12
22 and 18 months of age without *MYCN* amplification [68-70]. In addition, it was recently
23 showed that localized tumor with *MYCN* amplification and hyperdiploidy in this subgroup is
24 associated with better outcome [71, 72].

25 The application of a pan-genomic approach using neuroblastoma-specific PCR based
26 multiplex ligation-dependent probe amplification (MLPA) was recently validated in the
27 ongoing multicenter Low- and Intermediate-Risk Neuroblastoma Study (LINES), which is
28 organized by the International Society of Pediatric Oncology European Neuroblastoma
29 SIOPEX [73]. The treatment stratification concept in LINES is based on the results of recent
30 trials, which have suggested that the risk of relapse in patients who have low-risk tumors but
31 no *MYCN* amplification may well be defined by the presence versus absence of any
32 structural genetic abnormalities [47, 74, 75]. Several groups have made efforts to propose
33 mRNA expression-based classifiers for treatment stratification, which are in part combined
34 with CGH or microRNA [76-81]. The lack of overlap in the proposed gene lists of these
35 classifiers may be partly explained by the use of different patient cohorts, technologies
36 and/or bioinformatics approaches in the cited studies. However, it may just as well lend
37 support to the hypothesis that relapse or treatment failure in neuroblastoma is the result of
38 separate aberrant biological pathways in tumor pathogenesis. The use of mRNA- or
39 microRNA-based classifiers for outcome prediction and treatment stratification need to be
40 validated in prospective clinical trials.
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46 **Clinical characteristics and diagnostic work-up**

47 Due to their origin, neuroblastomas and the related GNBs and GNs develop in the adrenal
48 medulla or along the paravertebral chain and sympathetic ganglia in the abdomen, thorax,
49 pelvis, or neck. The majority of these tumors are located in the abdomen (65%), and more
50 thoracic and cervical primary tumors are found in younger children. In a minority of the cases
51 (around 1%), the primary site cannot be determined with certainty, because the tumor arises
52 at two or several sites simultaneously, or, alternatively, it infiltrates several organs in the
53 abdomen. The presentation at diagnosis ranges from a coincidentally detected painless
54 mass to a rapidly growing and expansive tumor that give rise to life-threatening symptoms.
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57 Cervical neuroblastomas are often seen with Horner's syndrome (ptosis, miosis, and
58 enophthalmos) and heterochromia. Tumors in the upper mediastinum can cause respiratory
59 distress as well as Horner's syndrome, whereas those occurring in the middle and lower
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mediastinum are usually asymptomatic and might be discovered by routine chest X-ray. Approximately half of all patients have disseminated disease at the time of diagnosis, and the sites most frequently involved are the bone marrow, skeleton, liver and lymph nodes, and less often the lungs and central nervous system. Disseminated disease is usually associated with unspecific symptoms, including fever, pallor, anorexia, and bone pain with subsequent mood changes and refusal to walk. Retro-orbital and orbital metastases are rather common, and produce a typical appearance of proptosis and periorbital ecchymoses. Growth into the foramina of the vertebra with compression of the spinal cord is seen mostly in small children with localized tumors [82, 83] and it is still not clear what treatment is best to avoid significant neurological complications in these patients.

The paraneoplastic opsoclonus myoclonus syndrome (OMS) is characterized by multidirectional rapid eye movement (opsoclonus), myoclonus, and brainstem ataxia and it occurs in 1–2 % of all neuroblastoma patients, particularly in localized cases. The exact mechanisms of this autoimmune reaction and the reasons for an association with severe neurological outcome are not yet known [84, 85]. The symptoms can precede the detection of a tumor mass by several months, and they may improve upon removal of the primary tumor. Many patients benefit from immunosuppressive treatment with rituximab and/or cyclophosphamide, which are currently being tested in clinical trials [86, 87].

Diagnosis of neuroblastoma is based on the following: a) an increase in catecholamines and catecholamine metabolites in the urine and/or serum; b) an unequivocal histological diagnosis of a tumor specimen or bone marrow aspirate/trephine with or without immunohistochemistry [88]. About 5-10% of the tumors do not produce catecholamines, and for these lesions, a panel of immunohistochemical stainings with positivity for neurofilaments, synaptophysin, Gap-43, neuron specific enolase (NSE) and additional markers can differentiate neuroblastoma from the other small blue round cell tumors found in children. Prior to treatment stratification, tumor sampling is done to achieve histological diagnosis and molecular analysis for identification of tumor subtypes of varying aggressiveness, and a clinical staging procedure is performed that includes CT/MRT of the primary tumor and a skeletal scan, a bone marrow aspiration/trephine biopsy, and a ¹²³I-MIBG (metaiodobenzylguanidine) scan to detect of potential metastases. Standardized techniques of the investigations and interpretation of the results are required in clinical trial protocols [89], and new guidelines were recently proposed for the detection of minimal residual disease (MRD) in blood and bone marrow [90]. Many centers now use true-cut biopsies instead of surgical biopsies in unresectable and metastatic cases, and this trend is justified if appropriate tissue material is secured for morphological and molecular diagnosis and tumor banking. An international consensus on tumor work-up and banking and standard operating procedures for molecular analysis of neuroblastoma tumor tissue was recently published to facilitate interpretations of future clinical and translational research [91].

Treatment stratification and prognostic factors

Tumor stage according to the revised and widely adopted International Neuroblastoma Staging System (INSS, Table 1) is based on the age of the patient at diagnosis, local and distant extent of the disease, and the resectability of the tumor [88]. INSS stage 1, 2A, 2B, and 3 are localized tumors of increasing local extension, whereas stage 4 is defined as distant metastatic disease. Stage 4S indicates children < 1 year of age who have metastases confined to the liver and the skin, and a maximum of 10% tumor cells in the bone marrow.

Over the last 15-20 years, INSS stage, patient age and amplification of *MYCN* have been used uniformly as the three major prognostic factors for treatment stratification in clinical trials worldwide. These parameters define at least two different patterns of disease. The first of these is neuroblastoma that arises during the initial months of life, with some patients showing spontaneous regression of the disease and most having excellent survival after minimal treatment. The second pattern differs markedly from the first, in that an unfavorable

1 outcome is expected in children who have *MYCN*-amplified tumors or are older than 18
2 months when diagnosed with metastatic tumors. Between these two extremes in the clinical
3 course, there are less well-defined groups with intermediate characteristics. It is plausible
4 that additional prognostic markers, such as histopathological findings, chromosomal
5 aberrations, and gene- or expression-level anomalies identified in molecular profiling can
6 help establish prognoses and consequently enable physicians to tailor different treatment
7 strategies to patients in the intermediate patient subgroup.

8 Tumor pathology according to the Shimada classification system has been used consistently
9 for risk stratification in the United States but not in all trials performed in Europe and other
10 parts of the world. The same is true for tumor cell ploidy and 1p deletion. Serum levels of
11 LDH, ferritin, and NSE have proven to predict event-free survival (EFS) and overall survival
12 (OS) in certain subgroups of neuroblastoma [92-94], and these parameters along with urine
13 and serum catecholamine metabolites, are mainly used as a marker of disease activity
14 during treatment and follow-up.
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18 Due to the use of slightly different variables used, risk grouping has not been uniform in the
19 various collaborative clinical trials around the world, which has complicating the comparison
20 and interpretation of the results obtained. To address this problem, the International
21 Neuroblastoma Risk Group (INRG) Task Force was created, which includes multidisciplinary
22 experts from major pediatric oncology groups in North America, Europe, Australia, and
23 Japan. The goal was to facilitate the comparison of risk-based clinical trials conducted in
24 different parts of the world by defining homogeneous pretreatment patient cohorts.
25

26 The INSS stage of locoregional tumors is based on the degree of surgical resection, and thus
27 it might differ greatly depending on the expertise of the local surgeon. Accordingly, a new
28 surgery-independent INRG Staging System was developed by the INRG Task Force [95, 96].
29 The premise is that a staging system based on preoperative, diagnostic images will be more
30 robust and reproducible than one based on operative findings and approaches. Since the
31 surgical risk factors are deduced from radiographic images, the term, "image-defined risk
32 factors" (IDRFs), was chosen, and a consensus was reached for the IDRFs (Table 2). The
33 INRG staging system defines four stages, which are designated: L1, L2, M, and MS; L
34 stands for localized, M for metastatic, and S for special, and 1 and 2 respectively denote with
35 and without surgical risk factors (Table 3).
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38 The INRG Task Force has subsequently developed the (above mentioned) INRG
39 Classification System (Table 4) to establish an international consensus approach for current
40 pretreatment risk stratification. In this effort, the prognostic effect of 13 variables was
41 analyzed in a cohort of 8800 patients diagnosed with neuroblastoma between 1990 and 2002
42 was analyzed, and a schema was developed that comprises four main prognostic groups
43 (very low risk, low risk, intermediate risk and high risk) and 16 pretreatment designations [7].
44 The age cut-of has been changed to 18 months from previous 12 months [3, 70, 97]. During
45 an immediate transitional period, the collaborative groups will gradually incorporate the new
46 INRG staging and classification system for treatment stratification into their new clinical trials.
47 This approach will greatly facilitate the comparison of risk-based trials conducted in different
48 parts of the world.
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51 **Current treatment modalities**

52 ***Neuroblastoma "wait-and-see" approach***

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54 A large group of INSS stage 4S neuroblastomas can regress spontaneously and patients
55 without symptoms and/or unfavorable prognostic markers are observed closely. Based on
56 clinical observations and case reports describing localized tumors with spontaneous
57 regression of macroscopic residual tumor tissue after incomplete surgery, it has been
58 suggested that either spontaneous differentiation or apoptosis can occur even in a subgroup
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1 of localized cases. The first prospective clinical trial randomizing between a “wait-and-see”
2 approach versus surgery for INSS stage 1 and 2 (without unfavorable prognostic markers)
3 showed that 47% of the tumors regressed spontaneously [5]. Also, in another prospective
4 study of neuroblastoma cases detected by mass screening, it was found that only 17 of 53
5 patients required any treatment [98]. Along with the steadily improving precision of risk
6 classification, it is very likely that use of the observational approach for localized tumors will
7 increase in future trials.

8 **Neuroblastoma surgery**

9 Surgery remains one of the cornerstones of neuroblastoma treatment. The goals of primary
10 surgery are to achieve the following: confirm the diagnosis; acquire tissue samples for

11
12 histological and molecular classification; resect the tumor with minimal morbidity. In patients
13 presenting with localized disease, surgery is the treatment of choice, if the anatomical
14 characteristics indicate that surgical resection is feasible. However, in some patients,
15 surgical risk factors are detected, and it is known that complications rise with increasing
16 attempts to complete surgery [99, 100]. In such cases it is sometimes necessary to use pre-
17 surgical chemotherapy to shrink the tumor before resection and to reduce the complication
18 rate. In contrast to the pivotal role of surgical treatment of localized neuroblastoma, the
19 suitability of this method for metastatic disease is somewhat controversial. Due to the high
20 incidence of local relapse in patients with metastatic disease, most high-risk treatment
21 protocols recommend surgical resection of the primary tumor after induction. The impact of
22 complete surgery for outcome is a matter of debate, and further investigation of this issue is
23 needed [100, 101].

24 **Neuroblastoma chemotherapy**

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Chemotherapy has an important role in the treatment of neuroblastoma, because the
majority of patients present with metastatic or locally advanced disease at diagnosis and
require systemic treatment. Alkylating agents (i.e., cyclophosphamide, iphosphamide,
busulfan, and melphalan), platinum analogues (i.e., cis-platinum and carboplatinum), vinca-
alkaloids (i.e., vincristine), epipodophyllotoxins (i.e., VP16, VM26), and anthracyclines (i.e.,
doxorubicin) have well-established activities and efficacies against neuroblastoma, and are
considered standard options. Over the last few years, a number of other agents, such as
topotecan, irinotecan, and temozolomide have also proven to be effective, and combinations
including these drugs are being tested in ongoing phase II studies [102-105]

The choice of type and dose of treatment given to patients with *intermediate-risk disease* has
varied between different collaborative groups. Survival is nearly 90% in these cohorts, and
thus the challenge is to identify patients for whom it might be possible to further reduce
therapy. Surgical resection and moderate-dose, multi-agent chemotherapy with
cyclophosphamide, cisplatin, carboplatin, etoposide, or doxorubicin constitutes the backbone
of treatment.

Treatment of *high-risk neuroblastoma patients* (i.e., all those with INSS stage 4 and > 12
months of age, and those in INSS stage 2, 3 and 4S with *MYCN* amplification) are divided
into the following: dose-intensive induction aimed at reducing the tumor burden;
consolidation treatment intended to remove the residual tumor and metastases; and
maintenance treatment designed to elimination of minimal residual disease. The induction
treatment consists of combinations of the same chemotherapeutic drugs as used in patients
with intermediate-risk disease but given at higher doses and with addition of vincristine.
Topotecan is randomized during the induction in a phase III study in ongoing German and
US trials, and this drug is also investigated after the pan-European high-risk induction if the
initial treatment response is insufficient. High-dose myeloablative chemotherapy with various
combinations of busulfan, melphalan, carboplatin, and etoposide followed by autologous
stem cell (PBSC) rescue is presently being used as consolidation treatment in most ongoing
high-risk trials and has been shown to improve outcome [106-108]. High dose treatment with
cyclophosphamide, etoposide, and melphalan (CEM) is currently randomized against a

1 combination of busulfan and melphalan in Europe [2], whereas in the United States CEM is
2 given randomized against tandem thiotepa/cyclophosphamide followed by reduced CEM
3 [109, 110].

4 **Radiation Therapy**

5
6 Neuroblastomas are radiosensitive, and tumoricidal doses range from 15 to 32 Gy depending
7 on the site and volume of the tumor, and the age of the patient. However, most collaborative
8 groups do not include external beam radiotherapy (EBRT) in the treatment of low- and
9 intermediate-risk patients, except for cases in which disease progresses despite
10 chemotherapy and surgery. In most trial involving high-risk patients, radiotherapy is given to
11 the site of the primary tumor during the consolidation phase. EBRT is also beneficial as
12 palliative care of painful sites. Total body irradiation (TBI) has been used in many of the
13 previous high-dose regimens, but, due to the late complications of such treatment, TBI is
14 currently being replaced by effective chemotherapeutic approaches [2]. Some attempts to
15 minimize late complications of EBRT by administering intraoperative radiotherapy to the
16 primary tumor have resulted in fewer late complications and better local control [111].

17
18 A radio-metabolic therapy for patients with INSS stages 3 and 4 neuroblastomas utilizes ¹³¹I-
19 labeled benzylguanidine (¹³¹I-MIBG); MIBG is a noradrenalin analogue that is incorporated
20 into the neurosecretory granules of neuroblastoma cells. Unfortunately, the use of this
21 therapeutic approach is limited to selected treatment centers due to dosimetry problems, the
22 toxicity ¹³¹I-MIBG, and non-homogeneous uptake by the tumors. Some groups have used
23 radio-metabolic therapy as first-line treatment, but long-term follow-up have indicated that
24 results were not favorable in those cases [112]. Other approaches have included ¹³¹I-MIBG in
25 the conditioning phase prior to hematopoietic stem cell transplantation, an approach that
26 probably increases in future high-risk protocols [113, 114].

27 **Maintenance therapy – treatment of minimal residual disease**

28
29 The majority of patients with high-risk disease respond to the induction and consolidation
30 treatment, but they also often experience local or systemic relapse attributable to minimal
31 residual disease (MRD). Therefore, much attention has been focused on maintenance
32 treatment consisting of biological therapies that include differentiation-inducing agents such
33 as retinoid derivatives or immunotherapy with IL-2 monoclonal antibodies. Retinoids are a
34 class of compounds that induce terminal differentiation of neuroblastoma cells *in vitro*.
35 Today, 13-cis retinoic acid given 2 weeks per month over 6 months post-transplantation is
36 part of most high-risk protocols, and this choice has been made because a randomized
37 phase III trial conducted by the Children's Oncology Group (COG) proved effect on EFS with
38 acceptable toxicity [1, 115]. Fenretinide, a synthetic variant retinoid, is now in phase II clinical
39 trials and may come to complement the treatment that is currently used [116, 117].

40
41 The profound immunosuppression produced by high-dose chemotherapy regimens creates
42 unfavorable conditions for application of active immunotherapy, but despite that, the use of
43 passive immunotherapy is feasible. Disialoganglioside (GD2) is a surface glycolipid antigen
44 present on neuroblastoma cells. Expression of GD2 in normal tissues is restricted to neurons
45 that are protected from the effects of intravenous monoclonal antibodies by the blood-brain
46 barrier. Therapies using various anti-GD2-antibodies have been assessed in phase I and
47 phase II trials, and their safety profile has been established. After a series of reports
48 concerning effect on survival, the first results of a randomized clinical trial of the chimeric
49 GD2 antibody ch14.18 in combination with IL-2 and GM-CSF were recently published and
50 indicated a 2-year EFS of 66% compared to 46% in favor of the treatment [114, 118-120].
51 Furthermore, the human variant hu14:18-IL2 is currently being tested [121]. The results
52 regarding the ch14.18 regimen suggest that the toxicity profile (including pain, allergic
53 reactions, and vascular leakage syndrome) is manageable and that this treatment will
54 successively be introduced to the majority of high-risk patients.

FUTURE TREATMENT ACCORDING TO THE INRG CLASSIFICATION SYSTEM (Table 4)

Very low-risk groups

The INRG classification system includes three very low-risk groups. They have no genetic aberrations of *MYCN* or 11q: INRG stages L1/L2, GNB intermixed and maturing GN, patients of all ages; INRG stage L1, any histological grade and patients of all ages; INRG stage MS, patients < 18 months. L1 patients will be treated with surgery only, or as stage MS, closely observed without any treatment in future protocols. Historically, most of these INSS stage 1 and 2 tumors have an excellent prognosis, with an overall survival close to 100% [122, 123].

Low-risk groups

There are three INRG low-risk groups (see Table 4): INRG stage L2, patients < 18 months with no *MYCN* amplification or 11q del; INRG stage L2, patients \geq 18 months with GNB nodular or differentiating histology; INRG stage M, patients < 18 months with hyperdiploid tumors. The use of close observations with “wait-and-see” strategy is expected to increase in some of these groups and further reduction of moderate dose-intensive chemotherapy will be carefully tested in clinical trials [6, 11, 124]. Based on the evidence from their own experience, the collaboration groups will use additional prognostic factors identified in their own cohorts for refined treatment decisions. Local recurrences can be managed by a second resection and metastatic relapse has proven to be curable by chemotherapy [125].

Intermediate-risk groups

There are four intermediate-risk groups in the INRG system, all without *MYCN* amplification: INRG stage L2, patients < 18 months with 11q del; INRG stage L2, patients \geq 18 months with undifferentiated or poorly differentiated histology; INRG stage M, patients < 12 and 12 to < 18 months with diploid tumors. These patients will be treated with moderate dose-intensive chemotherapy, which will be partly tailored according to response and surgical resectability of stage L2 tumors. Further reduction of treatment will be carefully tested in subgroups.

High-risk groups

The high-risk groups with *MYCN* amplification in all INRG stages, stage M \geq 18 months, and stage MS with 11q del will receive intensive induction chemotherapy, surgery, radiotherapy, myeloablative consolidation therapy with stem-cell rescue, and maintenance therapy for minimal residual disease with retinoids. Treatment with ch14.18 and ^{131}I -MIBG will subsequently be administered in the centers equipped for this treatment. To achieve further improvement, inclusion of new drugs that are based on the results from phase II studies will be included in randomized trials for high-risk patients.

Treatment of recurrent disease

Children who suffer local relapse of low- and intermediate-risk disease can benefit from further conventional treatment including second surgery with or without moderately intensive chemotherapy.

Recurrence of high-risk neuroblastoma is still extremely difficult to treat, and at present there is no broadly effective regimen that offers long-term cure [126]. However, potentially active agents have been identified in controlled clinical trials involving such cases, and some agents have resulted in long-term survival of small subsets of these patients. During the last years, there has been an increasing number of reports concerning phase I/II trials with partial or even complete responses in several patients (recently reviewed in [127, 128]). This trend is expected to continue as findings emerge from high-throughput research approaches and help to facilitate molecular characterization of individual tumors and identification of promising novel targets for treatment. Recently published results of early clinical trials involving treatment of recurrent neuroblastoma are briefly summarized in Table 5.

Novel drugs

1 Neuroblastoma research that include next-generation sequencing technologies to achieve
2 further molecular characterization of tumors from high-risk patients will no doubt disclose
3 potential targets for developing novel therapies to combat the most aggressive forms of the
4 disease. The availability of molecular inhibitors of relevant tyrosine kinase receptor pathways
5 presents an important translational opportunity to test these agents in children with high-risk
6 neuroblastoma. There is a complex interrelationship between receptor pathway members,
7 and hence inhibition at one point often induces feedback activation of other signaling
8 pathways, which illustrate the need to test these agents in combination.
9

10 In light of the frequency and importance of *MYCN* amplification in the pathogenesis of
11 neuroblastoma, blockade of MYCN signaling represents an important approach for the
12 development of new therapeutics. Inasmuch as there are no specific *myc* inhibitors are
13 available today, the most direct way to block *MYCN* is to use RNAi-based strategies.
14 However, although these methods are extremely useful in the laboratory, they have not yet
15 reached the clinic, largely due to inefficient delivery *in vivo* [129]. Aurora kinase A represents
16 another suitable therapeutic target, since it plays critical roles in regulation of the cell cycle
17 and spindle assembly, and it contributes to the stabilization of phosphorylated and
18 ubiquitinated MYCN [130]. Expression of Aurora kinase A is a negative prognostic factor in
19 neuroblastoma [131]. New data regarding the functions of inhibitors of Aurora kinase A in
20 cancer treatment suggests that such agents may have unique characteristics that can be
21 exploited in the treatment of neuroblastoma. MYCN degradation is a downstream factor that
22 has a critical impact on efficacy of the PI3K/mTOR pathway, which implies that clinical
23 inhibitors of PI3K, mTOR or AKT should show activity against MYCN-driven neuroblastomas
24 (reviewed in [132-134]).
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28 Pharmacological inhibition of activated ALK may also represent a promising novel approach
29 for neuroblastoma treatment. Neuroblastoma cell lines that harbor activating *ALK* mutations
30 have been found to respond well to the ALK inhibitors NVP-TAE684 and PF-02341066 [40,
31 67], and this observation provides a strong molecular rationale using ALK-targeted treatment
32 in defined subsets of neuroblastoma patients.
33

34 HDAC inhibitors are an additional emerging class of encouraging new anticancer drugs.
35 Neuroblastoma is the first tumor entity in which expression of all eleven classical HDAC
36 family members has been investigated systematically [135]. In that work, expression of such
37 HDACs was detected, but HDAC8 was the only isozyme that was found to be significantly
38 correlated with advanced disease stage, age, unfavorable tumor histology, 11q aberration,
39 and poor survival [135]. Considering that HDAC8-selective inhibitors are now available, it is
40 possible that HDAC8 will prove to be a suitable drug target in neuroblastoma differentiation
41 treatment.
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Conclusions and Perspectives

45 Neuroblastoma has long been a challenging disease for both clinical and preclinical
46 researchers. The most important accomplishments concerning treatment of neuroblastoma
47 patients that have occurred over the past decade involve proof of the efficacy of anti-GD2
48 ch14.18, topotecan, and ¹³¹I-MIBG for treatment of high-risk and recurrent neuroblastoma.
49 Moreover, there is evidence of a high spontaneous regression or differentiation potential in
50 subgroups of localized tumors and probably also in metastatic disease in children < 18
51 months of age. This will no doubt enable further reduction of chemotherapeutic treatment
52 and increase the numbers of cases that can be managed by the “wait-and-see” approach.
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56 Present major challenges in neuroblastoma research are to further refine treatment
57 stratification and to elucidate the pathogenesis of the different types of neuroblastoma as a
58 basis for identifying new treatment modalities focused on high-risk disease. Novel high-
59 throughput techniques have already provided molecular markers that can characterize both
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1 tumor behavior and patient outcome with fairly high accuracy. If prospective studies can
2 confirm the anticipated prognostic value of these markers, patients may profit from more
3 accurate risk assessment achieved by integrating these markers into the clinical routine.
4 Current high-throughput investigations primarily involve tumors and blood samples from
5 retrospective patient cohorts, and an appropriate clinical classification of the patients
6 regarding previous and present risk groups and staging systems are crucial for correct
7 interpretation of the data.

8 The discovery of other tumor-initiating events, like the recently revealed oncogenic mutations
9 of *ALK*, will aid further elucidation of neuroblastoma pathogenesis. Such knowledge, together
10 with novel information on altered signaling pathways in aggressively growing tumors, will
11 help to establish therapeutic strategies that specifically target key molecular factors in the
12 progression of neuroblastoma.
13

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20 **Conflict of interest**

21 The authors declare that there are no conflicts of interest.
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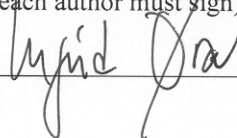
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Table 1 International Neuroblastoma Staging System [88]

Stage 1	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached and removed with the primary tumor may be positive)
Stage 2A	Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically
Stage 2B	Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically
Stage 3	Unresectable unilateral tumor infiltrating across the midline ^a , 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
Stage 4	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined for stage 4S)
Stage 4S	Localized primary tumor (as defined for stage 1, 2A, or 2B), with dissemination limited to skin, liver and/or bone marrow ^b (limited to infants <1 year of age)
Multifocal primary tumors (e.g., bilateral adrenal primary tumors) should be staged according to the greatest extent of disease, as defined previously, followed by subscript "M".	
^a The midline is defined as the vertebral column. Tumors originating on one side and "crossing the midline" must infiltrate to or beyond the opposite side of the vertebral column.	
^b Marrow involvement in stage 4S should be minimal, that is, less than 10% of total nucleated cells identified as malignant on bone marrow biopsy or on marrow aspirate. More extensive marrow involvement would be considered to be stage 4. The MIBG scan (if done) should be negative in the marrow.	

Table 2 Image-Defined Risk Factors in Neuroblastic Tumors [95]

<ul style="list-style-type: none"> § Ipsilateral tumor extension within two body compartments <ul style="list-style-type: none"> Neck-chest, chest-abdomen, abdomen-pelvis
<ul style="list-style-type: none"> Neck <ul style="list-style-type: none"> Tumor encasing carotid and/or vertebral artery and/or internal jugular vein Tumor extending to base of skull Tumor compressing the trachea
<ul style="list-style-type: none"> Cervico-thoracic junction <ul style="list-style-type: none"> Tumor encasing brachial plexus roots Tumor encasing subclavian vessels and/or vertebral and/or carotid artery Tumor compressing the trachea
<ul style="list-style-type: none"> Thorax <ul style="list-style-type: none"> Tumor encasing the aorta and/or major branches Tumor compressing the trachea and/or principal bronchi Lower mediastinal tumor, infiltrating the costo-vertebral junction between T9 and T12
<ul style="list-style-type: none"> Thoraco-abdominal <ul style="list-style-type: none"> Tumor encasing the aorta and/or vena cava
<ul style="list-style-type: none"> Abdomen/pelvis <ul style="list-style-type: none"> Tumor infiltrating the porta hepatis and/or the hepatoduodenal ligament Tumor encasing branches of the superior mesenteric artery at the mesenteric root Tumor encasing the origin of the coeliac axis, and/or of the superior mesenteric artery Tumor invading one or both renal pedicles Tumor encasing the aorta and/or vena cava Tumor encasing the iliac vessels Pelvic tumor crossing the sciatic notch
<ul style="list-style-type: none"> Intraspinal tumor extension whatever the location provided that: <ul style="list-style-type: none"> More than one third of the spinal canal in the axial plane is invaded and/or the perimedullary leptomeningeal spaces are not visible and/or the spinal cord signal is abnormal
<ul style="list-style-type: none"> Infiltration of adjacent organs/structures <ul style="list-style-type: none"> Pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, and mesentery
<ul style="list-style-type: none"> Conditions to be recorded, but not considered IDRFs <ul style="list-style-type: none"> Multifocal primary tumors Pleural effusion, with or without malignant cells Ascites, with or without malignant cells
Abbreviation: IDRFs, image-defined risk factors.

Table 3 International Neuroblastoma Risk Group Staging System [95]

Stage	Description
L1	Localized tumor not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment
L2	Locoregional tumor with presence of one or more image-defined risk factors
M	Distant metastatic disease (expect stage MS)
MS	Metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow

NOTE. See text for detailed criteria. Patients with multifocal primary tumors should be staged according to the greatest extent of disease as defined in the table.

Table 4 International Neuroblastoma Risk Group (INRG) Consensus Pretreatment Classification [7]

INRG Stage	Age (months)	Histologic Category	Grade of Tumor Differentiation	MYCN	11q Aberration	Ploidy	Pretreatment Risk Group
L1/L2		GN maturing; GNB intermixed					A Very low
L1		Any, except GN maturing or GNB intermixed		NA			B Very low
				Amp			K High
L2	< 18	Any, except GN maturing or GNB intermixed		NA	No		D Low
					Yes		G Intermediate
	≥ 18	GNB nodular; neuroblastoma	Differentiating	NA	No		E Low
					Yes		H Intermediate
		Poorly differentiated or undifferentiated	NA			N High	
				Amp			
M	< 18			NA		Hyperdiploid	F Low
	< 12			NA		Diploid	I Intermediate
	12 to < 18			NA		Diploid	J Intermediate
	< 18			Amp			O High
	≥ 18						P High
MS	< 18			NA	No		C Very low
					Yes		Q High
					Amp		R High

GN, ganglioneuroma; GNB, ganglioneuroblastoma; Amp, amplified; NA, not amplified; L1, localized tumor confined to one body compartment and with absence of image-defined risk factors (IDRFs); L2, locoregional tumor with presence of one or more IDRFs; M, distant metastatic disease (except stage MS); MS, metastatic disease confined to skin, liver and/or bone marrow in children < 18 months of age.

Table 5 Recent published phase I/II clinical trials in recurrent neuroblastoma

Response agent			Limited or no response agent		
agent	phase	reference	agent	phase	reference
lestauribin (anti TrkB)	I	Minturn JE <i>et al</i> 2011 [136]	emcitabine/oxaliplatin	II	Georger B <i>et al</i> 2011[137]
carboplatin-/irinotecan/ temozolomide	II	Kushner BH <i>et al</i> 2011 [103]	cyclophosphamide/ irinotecan/vincristine	II	Kushner BH <i>et al</i> 2011 [138]
irinotecan/temozolomide	II	Bagatell R <i>et al</i> 2011 [104]	decitabine (demethylating agent)	I	George R <i>et al</i> 2010 [139]
nifurtimox (<i>antiprotozoa</i>)	I	Saulnier <i>et al</i> 2011 [140]	oxaliplatin		Beaty O <i>et al</i> 2010 [141]
topotecan/temozolomide	I	Rubie H <i>et al</i> 2010 [105]	ixabepilone (microtubule inhibitor)	II	Jacobs S <i>et al</i> 2010 [142]
topotecan/cyclophosphamide versus topotecan	II	London WB <i>et al</i> 2010 [143]	rebeccamycin (topoisomerase)	II	Langevin AM <i>et al</i> 2008 [144]
zoledronic acid (bisphosphonate)	I	Russell HV <i>et al</i> 2010 [145]	irinotecan	II	Vassal G <i>et al</i> 2008 [146]
vorinostat (HDAC-inhib)/ 13-cis retinoid acid	I	Fouladi M <i>et al</i> 2010 [147]	imatinib	II	Bond M <i>et al</i> 2008 [148]
90Y-DOTATOC somatostatin analog, radionuclide	I	Menda Y <i>et al</i> 2010 [18]	erlotinib (EGFR-inhibitor) /temozolomide	I	Jakacki RI <i>et al</i> 2008 [149]
ramucirumab (anti-VEGFR-2)	I	Sprattlin JL <i>et al</i> 2010 [150]	tumor cell vaccine	I	Russel <i>et al</i> 2007 [151]
cediranib (anti-VEGFR)	I	Fox E <i>et al</i> 2010 [152]			
ABT-751 (β -tubulin inhibitor)	I	Fox E <i>et al</i> 2010 [153]			
PSC 833 (glycoprotein inhibitor)	I	Pein F <i>et al</i> 2007 [154]			
haploidentical SCT	I	Toporski J <i>et al</i> 2009 [155]			
¹³¹ I-MIBG (methaiodobenzoguanine)	I	Matthay KK <i>et al</i> 2009 [113]			
paxitacel/ifosfamide	I	Geller JI <i>et al</i> 2009 [156]			
topotecan/etoposide /cyclophosphamide	II	Simon T <i>et al</i> 2007 [157]			
17-AAG (17-N-Allylamino-17- demethoxygeldanamycin)	I	Bagatell R <i>et al</i> 2007 [158]			