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

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

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

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

**History of Neuroblastoma**

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

Epidemiology

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

The tumor is thought to arise from neural crest-derived cells that form the developing sympathetic nervous system in the embryo and fetus and are often described as being arrested at an early stage of differentiation. After migrating from the neural crest, the pluripotent sympathogonia form the sympathetic ganglia, the chromaffin cells of the adrenal medulla and the paraganglia, which represent the typical locations of neuroblastomas [30]. Neuroblastomas belong to the “small blue round cell” neoplasms of childhood and to the group of peripheral neuroblastic tumors (pNTs) [31], which includes neuroblastomas (NB), ganglioneuroblastomas (GNB) and the benign ganglioneuromas (GN). These tumor types reflect different degrees of maturation, ranging from undifferentiated cells with large dense nuclei and scant cytoplasm to poorly differentiated and differentiating cells, and finally to ganglion cells with inclusion of neurophils and Schwann cells with increased maturation.

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

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

Neuroblastoma predisposition and genetics

Familial neuroblastoma

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

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

**Sporadic neuroblastoma**

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

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

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

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

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

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

Somatic and activating mutations of the ALK gene were recently identified in approximately 8% of neuroblastoma tumors, and this constitutes a breakthrough in understanding of the pathogenesis of this disease [39, 40, 66, 67]. Interestingly, the spectra of somatic and germ-line ALK mutations differ. The existence of a link between such aberration and tumor biology has not yet been fully determined, since the studies published so far have revealed no consistent correlations between ALK mutations and aggressive neuroblastoma subtypes. Analysis of larger neuroblastoma series will provide further information about the precise relationship between the tumor phenotype and alterations in ALK mutations and/or genomic regions.

Early investigations demonstrated prognostic implication of ploidy (or DNA index) in neuroblastoma and this has been used for treatment stratification in several clinical trials in Germany and the United States. Studies have shown that in contrast to near-triploid tumors, near-diploid lesions constitute a risk factor for patients with metastatic disease between 12 and 18 months of age without MYCN amplification [68-70]. In addition, it was recently showed that localized tumor with MYCN amplification and hyperdiploidy in this subgroup is associated with better outcome [71, 72].

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

Clinical characteristics and diagnostic work-up

Due to their origin, neuroblastomas and the related GNBs and GNs develop in the adrenal medulla or along the paravertebral chain and sympathetic ganglia in the abdomen, thorax, pelvis, or neck. The majority of these tumors are located in the abdomen (65%), and more thoracic and cervical primary tumors are found in younger children. In a minority of the cases (around 1%), the primary site cannot be determined with certainty, because the tumor arises at two or several sites simultaneously, or, alternatively, it infiltrates several organs in the abdomen. The presentation at diagnosis ranges from a coincidentally detected painless mass to a rapidly growing and expansive tumor that give rise to life-threatening symptoms.

Cervical neuroblastomas are often seen with Horner’s syndrome (ptosis, miosis, and enophthalmos) and heterochromia. Tumors in the upper mediastinum can cause respiratory distress as well as Horner’s syndrome, whereas those occurring in the middle and lower
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 peri orbital 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
outcome is expected in children who have MYCN-amplified tumors or are older than 18 months when diagnosed with metastatic tumors. Between these two extremes in the clinical course, there are less well-defined groups with intermediate characteristics. It is plausible that additional prognostic markers, such as histopathological findings, chromosomal aberrations, and gene- or expression-level anomalies identified in molecular profiling can help establish prognoses and consequently enable physicians to tailor different treatment strategies to patients in the intermediate patient subgroup.

Tumor pathology according to the Shimada classification system has been used consistently for risk stratification in the United States but not in all trials performed in Europe and other parts of the world. The same is true for tumor cell ploidy and 1p deletion. Serum levels of LDH, ferritin, and NSE have proven to predict event-free survival (EFS) and overall survival (OS) in certain subgroups of neuroblastoma [92-94], and these parameters along with urine and serum catecholamine metabolites, are mainly used as a marker of disease activity during treatment and follow-up.

Due to the use of slightly different variables used, risk grouping has not been uniform in the various collaborative clinical trials around the world, which has complicating the comparison and interpretation of the results obtained. To address this problem, the International Neuroblastoma Risk Group (INRG) Task Force was created, which includes multidisciplinary experts from major pediatric oncology groups in North America, Europe, Australia, and Japan. The goal was to facilitate the comparison of risk-based clinical trials conducted in different parts of the world by defining homogeneous pretreatment patient cohorts.

The INSS stage of locoregional tumors is based on the degree of surgical resection, and thus it might differ greatly depending on the expertise of the local surgeon. Accordingly, a new surgery-independent INRG Staging System was developed by the INRG Task Force [95, 96]. The premise is that a staging system based on preoperative, diagnostic images will be more robust and reproducible than one based on operative findings and approaches. Since the surgical risk factors are deduced from radiographic images, the term, “image-defined risk factors” (IDRFs), was chosen, and a consensus was reached for the IDRFs (Table 2). The INRG staging system defines four stages, which are designated: L1, L2, M, and MS; L stands for localized, M for metastatic, and S for special, and 1 and 2 respectively denote with and without surgical risk factors (Table 3).

The INRG Task Force has subsequently developed the (above mentioned) INRG Classification System (Table 4) to establish an international consensus approach for current pretreatment risk stratification. In this effort, the prognostic effect of 13 variables was analyzed in a cohort of 8800 patients diagnosed with neuroblastoma between 1990 and 2002 was analyzed, and a schema was developed that comprises four main prognostic groups (very low risk, low risk, intermediate risk and high risk) and 16 pretreatment designations [7]. The age cut-off has been changed to 18 months from previous 12 months [3, 70, 97]. During an immediate transitional period, the collaborative groups will gradually incorporate the new INRG staging and classification system for treatment stratification into their new clinical trials. This approach will greatly facilitate the comparison of risk-based trials conducted in different parts of the world.

Current treatment modalities

Neuroblastoma “wait-and-see” approach

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

**Neuroblastoma surgery**

Surgery remains one of the cornerstones of neuroblastoma treatment. The goals of primary surgery are to achieve the following: confirm the diagnosis; acquire tissue samples for histological and molecular classification; resect the tumor with minimal morbidity. In patients presenting with localized disease, surgery is the treatment of choice, if the anatomical characteristics indicate that surgical resection is feasible. However, in some patients, surgical risk factors are detected, and it is known that complications rise with increasing attempts to complete surgery [99, 100]. In such cases it is sometimes necessary to use pre-surgical chemotherapy to shrink the tumor before resection and to reduce the complication rate. In contrast to the pivotal role of surgical treatment of localized neuroblastoma, the suitability of this method for metastatic disease is somewhat controversial. Due to the high incidence of local relapse in patients with metastatic disease, most high-risk treatment protocols recommend surgical resection of the primary tumor after induction. The impact of complete surgery for outcome is a matter of debate, and further investigation of this issue is needed [100, 101].

**Neuroblastoma chemotherapy**

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

**Radiation Therapy**

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

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

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

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

The profound immunosuppression produced by high-dose chemotherapy regimens creates unfavorable conditions for application of active immunotherapy, but despite that, the use of passive immunotherapy is feasible. Disialoganglioside (GD2) is a surface glycolipid antigen present on neuroblastoma cells. Expression of GD2 in normal tissues is restricted to neurons that are protected from the effects of intravenous monoclonal antibodies by the blood-brain barrier. Therapies using various anti-GD2-antibodies have been assessed in phase I and phase II trials, and their safety profile has been established. After a series of reports concerning effect on survival, the first results of a randomized clinical trial of the chimeric GD2 antibody ch14.18 in combination with IL-2 and GM-CSF were recently published and indicated a 2-year EFS of 66% compared to 46% in favor of the treatment [114, 118-120]. Furthermore, the human variant hu14:18-IL2 is currently being tested [121]. The results regarding the ch14.18 regimen suggest that the toxicity profile (including pain, allergic reactions, and vascular leakage syndrome) is manageable and that this treatment will 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 > 18 months with GNB nodular or differentiating histology; INRG stage M, patients < 18 months with hyperdiploid tumors. The use of close observations with “wait-an-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 > 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 > 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 131I-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 publish results of early clinical trials involving treatment of recurrent neuroblastoma are briefly summarized in Table 5.
Novel drugs

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

In light of the frequency and importance of MYCN amplification in the pathogenesis of neuroblastoma, blockade of MYCN signaling represents an important approach for the development of new therapeutics. Inasmuch as there are no specific myc inhibitors are available today, the most direct way to block MYCN is to use RNAi-based strategies. However, although these methods are extremely useful in the laboratory, they have not yet reached the clinic, largely due to inefficient delivery in vivo [129]. Aurora kinase A represents another suitable therapeutic target, since it plays critical roles in regulation of the cell cycle and spindle assembly, and it contributes to the stabilization of phosphorylated and ubiquitinated MYCN [130]. Expression of Aurora kinase A is a negative prognostic factor in neuroblastoma [131]. New data regarding the functions of inhibitors of Aurora kinase A in cancer treatment suggests that such agents may have unique characteristics that can be exploited in the treatment of neuroblastoma. MYCN degradation is a downstream factor which has a critical impact on efficacy of the PI3K/mTOR pathway, which implies that clinical inhibitors of PI3K, mTOR or AKT should show activity against MYCN-driven neuroblastomas (reviewed in [132-134]).

Pharmacological inhibition of activated ALK may also represent a promising novel approach for neuroblastoma treatment. Neuroblastoma cell lines that harbor activating ALK mutations have been found to respond well to the ALK inhibitors NVP-TAE684 and PF-02341066 [40, 67], and this observation provides a strong molecular rationale using ALK-targeted treatment in defined subsets of neuroblastoma patients.

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

Conclusions and Perspectives

Neuroblastoma has long been a challenging disease for both clinical and preclinical researchers. The most important accomplishments concerning treatment of neuroblastoma patients that have occurred over the past decade involve proof of the efficacy of anti-GD2 ch14.18, topotecan, and 131I-MIBG for treatment of high-risk and recurrent neuroblastoma. Moreover, there is evidence of a high spontaneous regression or differentiation potential in subgroups of localized tumors and probably also in metastatic disease in children < 18 months of age. This will no doubt enable further reduction of chemotherapeutic treatment and increase the numbers of cases that can be managed by the “wait-and-see” approach.

Present major challenges in neuroblastoma research are to further refine treatment stratification and to elucidate the pathogenesis of the different types of neuroblastoma as a basis for identifying new treatment modalities focused on high-risk disease. Novel high-throughput techniques have already provided molecular markers that can characterize both
tumor behavior and patient outcome with fairly high accuracy. If prospective studies can confirm the anticipated prognostic value of these markers, patients may profit from more accurate risk assessment achieved by integrating these markers into the clinical routine. Current high-throughput investigations primarily involve tumors and blood samples from retrospective patient cohorts, and an appropriate clinical classification of the patients regarding previous and present risk groups and staging systems are crucial for correct interpretation of the data.

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

Acknowledgements
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Conflict of interest
The authors declare that there are no conflicts of interest.

References


growth factor receptor inhibitor, in children and adolescents with refractory solid
Pharmacokinetics of orally administered ABT-751 in children with neuroblastoma and
study of oral PSC 833 combined with weekly intravenous etoposide in children with
iodine-131-metaiodobenzylguanidine with haploidentical stem cell transplantation and
posttransplant immunotherapy in children with relapsed/refractory neuroblastoma.
[156] Geller JI, Wall D, Perentesis J, Blaney SM, Bernstein M. Phase I study of paclitaxel
with standard dose ifosfamide in children with refractory solid tumors: a Pediatric
Topotecan, cyclophosphamide, and etoposide (TCE) in the treatment of high-risk
pharmacokinetic and pharmacodynamic study of 17-N-allylamino-17-
demethoxygeldanamycin in pediatric patients with recurrent or refractory solid tumors:
a pediatric oncology experimental therapeutics investigators consortium study. Clin
Cancer Res. 2007;13:1783-8.
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Author name: Ingrid Øra

Declarations

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Please state any competing interests

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Please state any sources of funding for your research

Swedish Childhood Cancer Foundation
Lund University Hospital Donation Funds

Signature (a scanned signature is acceptable, but each author must sign)

Ingrid Øra

Print name

Ingrid Øra
Table 1 International Neuroblastoma Staging System [88]

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

* 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”.
* The midline is defined as the vertebral column. Tumors originating on one side and “crossing the midline” must infiltrate to or beyond the opposite side of the vertebral column.
* Marrow involvement in stage 4S should be minimal, 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>
<thead>
<tr>
<th>Section</th>
<th>Defined Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck</td>
<td>Ipsilateral tumor extension within two body compartments</td>
</tr>
<tr>
<td></td>
<td>Neck, chest, chest-abdomen, abdomen-pelvis</td>
</tr>
<tr>
<td></td>
<td>Tumor encasing carotid and/or vertebral artery and/or internal jugular vein</td>
</tr>
<tr>
<td></td>
<td>Tumor extending to base of skull</td>
</tr>
<tr>
<td></td>
<td>Tumor compressing the trachea</td>
</tr>
<tr>
<td></td>
<td>Cervico-thoracic junction</td>
</tr>
<tr>
<td></td>
<td>Tumor encasing brachial plexus roots</td>
</tr>
<tr>
<td></td>
<td>Tumor encasing subclavian vessels and/or vertebral and/or carotid artery</td>
</tr>
<tr>
<td></td>
<td>Tumor compressing the trachea</td>
</tr>
<tr>
<td>Thorax</td>
<td>Tumor encasing the aorta and/or major branches</td>
</tr>
<tr>
<td></td>
<td>Tumor compressing the trachea and/or principal bronchi</td>
</tr>
<tr>
<td></td>
<td>Lower mediastinal tumor, infiltrating the costo-vertebral junction between T9 and T12</td>
</tr>
<tr>
<td>Thoraco-abdominal</td>
<td>Tumor encasing the aorta and/or vena cava</td>
</tr>
<tr>
<td>Abdomen/pelvis</td>
<td>Tumor infiltrating the porta hepatis and/or the hepatoduodenal ligament</td>
</tr>
<tr>
<td></td>
<td>Tumor encasing branches of the superior mesenteric artery at the mesenteric root</td>
</tr>
<tr>
<td></td>
<td>Tumor encasing the origin of the coeliac axis, and/or of the superior mesenteric artery</td>
</tr>
<tr>
<td></td>
<td>Tumor invading one or both renal pedicles</td>
</tr>
<tr>
<td></td>
<td>Tumor encasing the aorta and/or vena cava</td>
</tr>
<tr>
<td></td>
<td>Tumor encasing the iliac vessels</td>
</tr>
<tr>
<td></td>
<td>Pelvic tumor crossing the sciatic notch</td>
</tr>
<tr>
<td>Intraspinal tumor extension whatever the location provided that: 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</td>
<td></td>
</tr>
<tr>
<td>Infiltration of adjacent organs/structures</td>
<td>Pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, and mesentery</td>
</tr>
<tr>
<td>Conditions to be recorded, but not considered IDRFs</td>
<td>Multifocal primary tumors</td>
</tr>
<tr>
<td></td>
<td>Pleural effusion, with or without malignant cells</td>
</tr>
<tr>
<td></td>
<td>Ascites, with or without malignant cells</td>
</tr>
</tbody>
</table>

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

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>
<thead>
<tr>
<th>INRG Stage</th>
<th>Age (months)</th>
<th>Histologic Category</th>
<th>Grade of Tumor Differentiation</th>
<th>MYCN</th>
<th>11q Aberration</th>
<th>Ploidy</th>
<th>Pretreatment Risk Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1/L2</td>
<td></td>
<td>GN maturing; GNB intermixed</td>
<td>NA</td>
<td>Amp</td>
<td></td>
<td></td>
<td>A Very low</td>
</tr>
<tr>
<td>L1</td>
<td></td>
<td>Any, except GN maturing or GNB intermixed</td>
<td>NA</td>
<td>Amp</td>
<td></td>
<td></td>
<td>B Very low</td>
</tr>
<tr>
<td>L2</td>
<td>&lt; 18</td>
<td>GN maturing or GNB intermixed</td>
<td>NA</td>
<td>Amp</td>
<td></td>
<td></td>
<td>D Low</td>
</tr>
<tr>
<td>L2</td>
<td>≥ 18</td>
<td>GNB nodular; neuroblastoma</td>
<td>Poorly differentiated or undifferentiated</td>
<td>Amp</td>
<td></td>
<td></td>
<td>G Intermediate</td>
</tr>
<tr>
<td>M</td>
<td>&lt; 18</td>
<td>NA</td>
<td>Hyperdiploid</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>&lt; 12</td>
<td>NA</td>
<td>Diploid</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>12 to &lt; 18</td>
<td>NA</td>
<td>Diploid</td>
<td>J</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>≥ 18</td>
<td>NA</td>
<td>Amp</td>
<td>O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>&lt; 18</td>
<td>NA</td>
<td>No</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>≥ 18</td>
<td>NA</td>
<td>Amp</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Response agent</th>
<th>Limited or no response agent</th>
<th>phase</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>lestaurtinib (anti-TrkB)</td>
<td>emcitabine/oxaliplatin</td>
<td>I</td>
<td>George B et al 2011 [137]</td>
</tr>
<tr>
<td>carboplatin-irinotecan/temozolomide</td>
<td>cyclophosphamide/irinotecan/vincristine</td>
<td>II</td>
<td>Kushner BH et al 2011 [138]</td>
</tr>
<tr>
<td>irinotecan/temozolomide</td>
<td>decitabine (demethylating agent)</td>
<td>I</td>
<td>George R et al 2010 [139]</td>
</tr>
<tr>
<td>carboplatin-rinotecan/temozolomide</td>
<td>oxaliplatin</td>
<td>I</td>
<td>Beatty O et al 2010 [141]</td>
</tr>
<tr>
<td>topotecan/temozolomide</td>
<td>irinotecan</td>
<td>II</td>
<td>Jacobs S et al 2010 [142]</td>
</tr>
<tr>
<td>irinotecan</td>
<td>rebeccamycin (topoisomerase)</td>
<td>II</td>
<td>Langevin AM et al 2008 [144]</td>
</tr>
<tr>
<td>carboplatin (demethylating agent)</td>
<td>cyclophosphamide</td>
<td>I</td>
<td>Fouladi M et al 2010 [147]</td>
</tr>
<tr>
<td>90Y-DOTATOC somatostatin analog, radionuclide</td>
<td>imatinib</td>
<td>II</td>
<td>Bond M et al 2008 [148]</td>
</tr>
<tr>
<td>ramucirumab (anti-VEGFR-2)</td>
<td>erlotinib (EGFR-inhibitor)</td>
<td>I</td>
<td>Jakacki RI et al 2008 [149]</td>
</tr>
<tr>
<td>cediranib (anti-VEGFR)</td>
<td>vorinostat (HDAC-inhib) 13-cis retinoid acid</td>
<td>I</td>
<td>Russell HV et al 2010 [145]</td>
</tr>
<tr>
<td>ABT-751 (g-tubulin inhibitor)</td>
<td>zoledronic acid (bisphosphonate)</td>
<td>I</td>
<td>Menda Y et al 2010 [18]</td>
</tr>
<tr>
<td>PSC 833 (glycoprotein inhibitor)</td>
<td>vorinostat (HDAC-inhib) 13-cis retinoid acid</td>
<td>I</td>
<td>Menda Y et al 2010 [18]</td>
</tr>
<tr>
<td>haplodental SCT</td>
<td>TMZ</td>
<td>I</td>
<td>Toporski J et al 2009 [155]</td>
</tr>
<tr>
<td>131I-MIBG (methaiodobenzoguanine)</td>
<td>Taxol</td>
<td>I</td>
<td>Toporski J et al 2009 [113]</td>
</tr>
<tr>
<td>paxitacei/ifosfamide</td>
<td>topotecan/etoposide</td>
<td>II</td>
<td>Simon T et al 2007 [157]</td>
</tr>
<tr>
<td>topotecan</td>
<td>zoledronic acid (bisphosphonate)</td>
<td>I</td>
<td>Fouladi M et al 2010 [147]</td>
</tr>
<tr>
<td>17-AAG (17-N-Allylamino-17-demethoxygeldanamycin)</td>
<td>131I-MIBG (methaiodobenzoguanine)</td>
<td>I</td>
<td>Matthay KK et al 2009 [113]</td>
</tr>
</tbody>
</table>

Reference numbers are provided in square brackets [136-158].