Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Treatment of high-risk neuroblastoma in children: recent clinic trial results

Clin. Invest. (2013) 3(11), 1071–1081

Neuroblastoma is the most common extracranial solid tumor in childhood and accounts for 15% of all pediatric oncology deaths. Children with high-risk disease have a particularly poor prognosis, with nearly half succumbing to their disease despite aggressive multimodal therapy. A better understanding of the biological and clinical risk factors over the past few decades have led to significant breakthroughs and a series of clinical trials have improved outcomes for children with high-risk neuroblastoma. Despite this, many children will still die of their disease and novel approaches are desperately needed. The purpose of this review is to summarize the outcomes of recent clinical trials for children with high-risk neuroblastoma.

Keywords: autologous stem cell transplant • clinical trials • immunotherapy • neuroblastoma • novel therapies • radiation therapy • treatment

Emily Greengard*[1], Christine Hill-Kayser[2] & Rochelle Bagatell[3]

[1]Division of Pediatric Hematology/Oncology, University of Minnesota Amplatz Children's Hospital, D-557 Mayo Memorial Building, 420 Delaware St, SE, MMC 484, Minneapolis, MN 55455, USA

[2]Department of Radiation Oncology, The University of Pennsylvania, PA, USA

[3]Division of Oncology, The Children's Hospital of Philadelphia & The University of Pennsylvania, PA, USA

*Author for correspondence:
Tel.: +1 612 626 2778
Fax: +1 612 626 2815
E-mail: emilyg@umn.edu

Neuroblastoma is the most common extracranial solid tumor in childhood, accounting for more than 7% of malignancies in children younger than 15 years of age and 15% of all pediatric oncology deaths [1]. It is a heterogeneous malignancy with a broad spectrum of clinical behavior. Extensive clinical and basic research over the last four decades has shed light on the biology and treatment of neuroblastoma, but despite remarkable progress, important challenges remain. While some tumors may regress or differentiate spontaneously, others will metastasize widely and result in death despite aggressive multimodality therapy. Chemotherapy and radiotherapy resistance continue to present major therapeutic challenges.

Neuroblastoma risk stratification is based on age at diagnosis, disease stage, tumor histology and tumor biology. The current staging system uses clinical characteristics and imaging defined risk factors to stage the disease as L1, L2, M or MS [2]. Locoregional tumors are designated as either L1 or L2 based on the presence or absence of anatomic characteristics that would make complete surgical excision unsafe or impracticable at the time of diagnosis [3]. Metastatic tumors are defined as stage M, except in cases in which metastases are confined to the skin, liver and/or bone marrow in children younger than 18 months of age. These children are defined as having stage MS disease [2]. Biologic features associated with inferior outcome in patients with neuroblastoma include amplification of the *MYCN* oncogene, the presence of segmental chromosomal alterations, and diploidy; these tumor characteristics have been incorporated into the current international approach to risk assignment [4]. In general, children who have tumors with *MYCN* amplification are considered to have high-risk disease. While all children with metastatic disease who were over 12 months of age at the time of diagnosis were previously considered to have high-risk disease even in the absence of *MYCN* amplification, patients diagnosed between the ages of 12 and 18 months whose tumors have favorable biologic features have been shown to have more favorable outcomes than do older children [5].

FUTURE SCIENCE part of fsg

Currently, therefore, patients whose tumors are *MYCN* nonamplified are considered high risk if they are older than 18 months of age at diagnosis with stage M disease. Small subsets of patients <18 months of age may also be considered to have high-risk disease (Table 1) [2]. Despite the aggressive therapy that these children will receive, approximately half will eventually relapse and succumb to their disease. Clinical trials throughout the past decade have improved the outcomes for children with high-risk neuroblastoma; however, it is still evident that novel approaches are needed. The purpose of this review is to summarize the outcomes of recent clinical trials for children with high-risk neuroblastoma.

Current therapy for high-risk neuroblastoma

Current therapy for high-risk neuroblastoma is comprised of three main components. The induction phase includes multiagent chemotherapy and surgery; the consolidation phase consists of myeloablative chemotherapy with stem cell rescue followed by external beam radiation and the postconsolidation phase is designed to treat minimal residual disease, and includes both immunotherapy (such as the chimeric 14.18 antibody directed against the disialoganglioside GD2 augmented by granulocyte macrophage stimulating factor and IL-2 in North America, and the anti-GD2 antibody with IL-2 alone in Europe) and a differentiating agent (isotretinoin). This review describes recent clinical trial results for each phase of therapy for patients with high-risk neuroblastoma.

Recent clinical trial results: induction

The goal of the induction phase of therapy is to reduce overall disease burden. This is accomplished using intensive, multiagent induction chemotherapy followed by surgical intervention to achieve local control of the

Table 1. The International Neuroblastoma Risk Group staging system.

INRG stage	Age (months)	Histologic category	Grade of tumor differentiation	*MYCN*	11q aberration	Ploidy	Pretreatment risk group
L1/L2	Any	GN maturing GNB intermixed	Any	Any	Any	Any	Very low
L1	Any	Any, except GN maturing or GNB intermixed	Any	NA	Any	Any	Very low
L1	Any	Any, except GN maturing or GNB intermixed	Any	AMP	Any	Any	High
L2	<18	Any, except GN maturing or GNB intermixed	Any	NA	No	Any	Low
L2	<18	Any, except GN maturing or GNB intermixed	Any	NA	Yes	Any	Intermediate
L2	<18	GNB nodular, neuroblastoma	Differentiating	NA	No	Any	Low
L2	<18	GNB nodular, neuroblastoma	Differentiating	NA	Yes	Any	Intermediate
L2	≥18	GNB nodular, neuroblastoma	Poorly differentiated, undifferentiated	NA	Any	Any	Intermediate
L2	≥18	GNB nodular, neuroblastoma	Poorly differentiated, undifferentiated	AMP	Any	Any	High
M	<18	Any	Any	NA	Any	Hyperdiploid	Low
M	<18	Any	Any	NA	Any	Diploid	Intermediate
M	Any	Any	Any	AMP	Any	Any	High
M	≥18	Any	Any	Any	Any	Any	High
MS	<18	Any	Any	NA	No	Any	Very low
MS	<18	Any	Any	NA	Yes	Any	High
MS	<18	Any	Any	AMP	Any	Any	High

AMP: Amplified; GN: Ganglioneuroma; GNB: Ganglioneuroblastoma; INRG: International Neuroblastoma Risk Group; NA: Nonamplified.
Adapted from [4].

primary tumor. In North America, induction chemotherapy regimens being incorporated into current clinical trials are based on a series of studies conducted at Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY, USA). The N6 protocol developed at MSKCC alternated courses of cyclophosphamide, doxorubicin and vincristine with cisplatin and etoposide for a total of seven cycles. In a single institution study of the N6 induction regimen (n = 24), 87% of patients achieved a complete (CR) or very good partial (VGPR) response [6]. A reduction from seven to five cycles of chemotherapy (the N7 protocol) resulted in similar outcomes and less toxicity, with a CR/VGPR rate of 79%, again in a single-center trial [7].

Based on these results, the Children's Oncology Group (COG) incorporated a modified N6 regimen (six cycles of chemotherapy) into the high-risk neuroblastoma trial A3973. In the cooperative group setting, the end-induction CR + VGPR rate following this therapy was 52%. This was similar to results from a multicenter trial performed by the French Society of Pediatric Oncology, in which 21 out of 47 patients who received this induction regimen achieved CR at metastatic sites [8]. Data from both the COG A3973 and French Society of Pediatric Oncology trials suggest that chemotherapy resistance remains an obstacle. Hematopoietic and mucosal toxicity prevent further intensification of induction chemotherapy, leading investigators to study the addition of newer agents to further improve response rates. The safety and feasibility of the addition of two cycles of topotecan and cyclophosphamide prior to five cycles of N7 chemotherapy were demonstrated in the COG pilot study ANBL02P1 [9]. This induction regimen has recently been studied further in the Phase III COG study ANBL0532; results of this trial are expected in the near future.

In Europe, investigators have studied the concept of rapid administration of maximum tolerated doses of chemotherapy agents in order to induce more rapid cell death and decrease the opportunity for drug resistance. In 2008 Pearson *et al.* published the results of a trial in which chemotherapy was administered at 10-day intervals, alternating more myelosuppressive regimens (vincristine, carboplatin and etoposide, or vincristine, cyclophosphamide and etoposide) with less myelosuppressive regimens (vincristine and cisplatin) [10]. This rapid regimen (cisplatin, vincristine, carboplatin, etoposide and cyclophosphamide, known as rapid COJEC) was compared with a conventional regimen that utilized the same agents administered every 21 days in a randomized trial, the European Neuroblastoma Group Fifth Study. There was no difference in 3-year event-free survival (EFS) or overall survival (OS) when the two groups were compared; however differences in 5-year EFS were statistically significant (18% in the standard group and 30% in the rapid group; p = 0.02) [10]. In addition, myeloablative therapy was given a median of 55 days earlier in patients assigned to the rapid treatment than those assigned to standard treatment. As expected, infectious complications and time in the hospital were greater with rapid treatment [10].

To reduce the incidence of febrile neutropenia and infectious complications during rapid COJEC induction, a follow-up study, European HR-NBL1/SIOPEN, randomly assigned patients to primary prophylactic versus symptom-triggered GCSF. In this trial, patients on the prophylactic colony stimulating factor arm had significantly fewer febrile neutropenic episodes, days with fever, hospital days and antibiotic days. Rapid COJEC with prophylactic growth factor support is now the standard of care for induction therapy for newly diagnosed children with high-risk neuroblastoma in SIOPEN institutions [11].

Recent clinical trial results: consolidation

Myeloablative therapy with stem cell rescue was shown to improve EFS in children with high-risk neuroblastoma in the late 1990s and results were confirmed with long-term follow up 10 years later [12,13]. In the Children's Cancer Group 3891 study, patients were randomized to receive postinduction therapy with either continuation chemotherapy or total body irradiation (TBI) followed by carboplatin, etoposide and melphalan (CEM) and an infusion of stem cells derived from autologous bone marrow. Among patients assigned to autologous transplantation, 3-year EFS (from the time of randomization) was 34%, compared with 22% for patients assigned to continuation chemotherapy [13]. At 5 years, EFS was 30% for patients assigned to the transplant arm and 19% for patients assigned to continuation chemotherapy [14].

The German NB97 study also evaluated the use of myeloablative consolidation therapy in high-risk neuroblastoma. Patients were randomized to undergo either myeloablative therapy consisting of CEM, or continuation therapy (oral cyclophosphamide) following an intensive induction [15]. The difference in 3-year OS between the two groups did not reach the level of statistical significance in an intention to treat analysis (62% for the transplant group vs 53% for the continuation chemotherapy group; p = 0.09); however, the difference in 3-year EFS was significant (47 vs 31%; p = 0.02). Randomization was stopped due to the improved outcome in the transplant group and excessive toxicity in the continuation chemotherapy group. Importantly, the transplant preparative regimen in this trial included chemotherapy only, indicating that the favorable results of intensified therapy could be achieved without TBI.

Although patients randomized to peripheral blood stem cell transplant (ASCT) who had persistence of metaiodobenzylguanidine (MIBG) avid lesions at the end of induction therapy received therapeutic MIBG, this study nonetheless provided support for the concept of consolidation therapy without external beam TBI.

The results of the European Neuroblastoma Study Group-1 trial confirmed the finding that a non-TBI containing a transplant preparative regimen could be used in children with neuroblastoma [16]. Differences in survival between those who underwent transplant and those who received no additional therapy were not statistically significant for the cohort as a whole, however among children over 1 year of age with international neuroblastoma staging system stage 4 disease, an improvement in 5-year EFS in patients treated with melphalan and autologous stem cell rescue was observed (33 vs 17%; p = 0.01) [16]. Data comparing TBI-containing preparative regimens to chemotherapy-only regimens are limited. However, in recent years there has been increased recognition of late effects related to TBI in survivors, including growth abnormalities, cataracts, thyroid disease and second malignancies [17]. Results of a large (n = 4098) retrospective study indicate that there is no clear improvement in outcome attributable to inclusion of TBI in autologous transplant conditioning for children with neuroblastoma [18] and chemotherapy-only preparative regimens are now used in most cooperative group trials.

The chemotherapy-only preparative regimen most commonly used in North America, CEM, was initially evaluated in a limited institution study (91LA6). The 3-year EFS of 49% observed in patients who received CEM after achieving stable disease or better during induction (n = 71) led to further study of this preparative regimen in the context of the COG trial A3973 [19]. A total of 368 patients completed CEM conditioning for ASCT on A3973. Though the toxic death rate (3%) and the rate of renal failure requiring dialysis (<1%) were low and the 2-year EFS was 48%, CEM was associated with significant toxicity, including severe mucositis in nearly 75% of patients [20].

In Europe, other preparative regimens including busulfan-containing regimens have been studied more extensively. A multivariate analysis of retrospective data generated through the European Bone Marrow Transplant Registry suggested that busulfan-containing regimens were associated with improved outcomes [18]. Based on these data, the International Society of Paediatric Oncology European Neuroblastoma (SIOPEN) HR NBL-1 randomized Phase III study directly compared a busulfan-melphalan (BuMel) preparative regimen with CEM. Although only 598 out of the 1577 patients enrolled on the study underwent randomization, among those who were randomized the 3-year EFS for those assigned to receive BuMel was 49% compared with 33% for those assigned to CEM (p < 0.001). Relapse was less common among those randomized to BuMel rather than CEM, and OS at 3 years was higher among those randomized to the BuMel arm (61 vs 48%; p = 0.004) [21]. The incidence of oral mucositis, gastrointestinal toxicity, ototoxicity, infection and renal toxicity was lower among patients treated with BuMel compared with those treated with CEM. However, clinically relevant sinusoidal obstruction syndrome (SOS) occurred in 18% of patients on the BuMel arm compared with 4% on the CEM arm. Furthermore, outcomes for patients randomized to received CEM were inferior to those reported in other trials of this preparative regimen [20]. Thus, while BuMel has now become the standard transplant preparative regimen for SIOPEN centers, it is still being evaluated as a component of therapy in other cooperative groups.

Further intensification of therapy through use of sequential autologous transplants has also been studied. The LCME2 trial demonstrated that consecutive cycles of ASCT could be delivered and additional pilots demonstrated the feasibility of this approach [22–30]. The largest trial of tandem transplantation published to date included 97 patients, 82 of whom underwent two consecutive courses of myeloablative therapy (one TBI-containing preparative regimen and one chemotherapy-only preparative regimen) [31]. The 7-year progression-free survival and OS rates of 45 and 53% provided the impetus for a large randomized study of single versus tandem transplant through the COG (ANBL0532). The results of ANBL0532 will also inform current thinking about the role of myeloablative therapy in the context of current-era induction regimens as well as postconsolidation therapy.

Most early studies of stem-cell transplants in children with high-risk neuroblastoma were performed using autologous bone marrow as the stem cell source. Studies of allogeneic transplantation have also been performed, but concerns have been raised about transplant-related mortality (TRM) in the allogeneic setting. A retrospective study has shown that while TRM dropped from 11% prior to 1995 to 4% after this time in patients with neuroblastoma who underwent autologous transplant, the 16% incidence of TRM in neuroblastoma patients undergoing allogeneic transplantation was unchanged over this time period. Furthermore, 5-year progression-free survival was significantly higher in patients who underwent autologous rather than allogeneic transplantation in this cohort [18]. Reduced intensity conditioning has the potential to reduce allogeneic TRM, but further study is needed before allogeneic transplant becomes more widely used in children with neuroblastoma.

While bone marrow was used most widely in early studies of ASCT for neuroblastoma, a transition to the use of peripheral blood stem cells (PBSCs) was made after a series of studies demonstrated the feasibility of harvesting PBSC from small children [32–36]. Harvesting relatively early in induction therapy is recommended so that stem cells are less likely to have been affected by exposure to alkylators and epipodophyllotoxins [37]. Collections are particularly robust when stem cells are harvested after topotecan/cyclophosphamide as initial therapy [9]. In patients who did not undergo harvest with initial therapy, the CXC chemokine receptor type 4 inhibitor plerixafor has been used successfully in small series [38,39].

Concerns regarding contaminating neuroblastoma cells among harvested PBSCs led to studies of *ex vivo* purging of stem cell products. Positive selection of CD34-expressing cells permits retention of hematopoietic progenitor cells and removal of neuroblastoma cells [40]. However, CD34 selection can cause depletion of T cells and potentially alter immune recovery in patients. Epstein–Barr virus lymphoproliferative disease has been observed in patients who received CD34-selected stem cell products and concerns regarding an increased incidence of serious viral illnesses led to discontinuation of CD34 selection during the COG ANBL00P1 trial of tandem ASCT [30,41]. Negative selection to diminish tumor contamination of stem cell products appeared to be a promising approach based upon preclinical data [42]. However, when studied in a large, randomized cooperative group trial, immunomagnetic purging did not improve EFS. A total of 489 children with high-risk neuroblastoma were enrolled on the COG A3973 trial, and 244 patients received stem cell products that had undergone depletion of phagocytes followed by purging using five monoclonal antibodies. The 2-year EFS was 49% in the unpurged group and 47% in the purged group ($p = 0.788$) [20]. In the absence of improved outcomes in patients receiving purged PBSCs, standard practice no longer includes this additional step in stem cell processing.

Because neuroblastoma is a radiosensitive tumor and because TBI is no longer widely used during conditioning for ASCT, there is interest in the use of the targeted radionuclide ^{131}I-MIBG as a component of consolidation therapy for patients with high-risk neuroblastoma who have MIBG-avid disease. MIBG is a norepinephrine analog that is preferentially taken up by neuroblastoma cells. The myelosuppression associated with doses of above 12 mCi/kg can be clinically significant; stem cell support is often provided for patients following doses greater than this. Single agent ^{131}I-MIBG at a dose of 18 mCi/kg was associated with a high objective response rate of 37% in a Phase II trial for patients with relapsed or refractory neuroblastoma [43]. This effective but myelosuppressive therapy has been integrated into upfront therapeutic regimens for children with high-risk neuroblastoma in several studies. In total, 44 children were enrolled on a Dutch study of ^{131}I-MIBG as the first intervention in newly diagnosed patients with high-risk disease; 39 received at least two infusions of the radionuclide. The majority of these children (34/39) tolerated an interval of 4 weeks between infusions, and a 66% response rate was observed after the two cycles of therapy [44]. A Phase I study of ^{131}I-MIBG followed by CEM demonstrated that this therapy can be delivered to patients with refractory neuroblastoma in the upfront setting [45]. A single institution retrospective study of ^{131}I-MIBG followed by BuMel demonstrated the feasibility of this regimen in a small cohort of patients with refractory neuroblastoma. Of note, one of the eight patients on this study developed severe SOS resulting in death [46]. Given this observation, as well as the higher rate of SOS in the SIOPEN trial using BuMel conditioning, further evaluation of ^{131}I-MIBG followed by BuMel in a larger cohort of patients is warranted. A COG pilot study using this regimen for newly diagnosed patients (ANBL12P1) is ongoing. If the feasibility of this approach is confirmed, the COG will conduct a randomized trial of ^{131}I-MIBG and BuMel SCT.

External beam radiation as a component of consolidation

Although neuroblastoma is a systemic disease, external beam radiotherapy (EBRT) is a part of modern treatment regimens to address residual (gross or microscopic) disease at the primary tumor site and persistent disease after aggressive systemic treatment. Postoperative local/regional failure has been shown to impact OS, and optimal dose and technique for delivery of EBRT have been evaluated in the past decade [47]. In the CCG3891 trial, patients who had incomplete surgical resections of their primary tumors were nonrandomly assigned to receive EBRT. Patients who were to undergo autologous transplant received 10 Gy delivered to the primary site and subsequently received TBI as part of the transplant preparative regimen, bringing the total radiation dose to the primary tumor site to 22 Gy. Patients randomized to receive continuation chemotherapy were to receive 10 Gy to the primary site alone. EBRT was delivered only to patients who had residual tumor following surgery, and therefore it was not surprising that administration of EBRT did not have a statistically significant impact on local recurrence rates either among the overall population of patients who underwent transplant or those who received continuation chemotherapy. However, among the cohort of patients who received EBRT due to the presence of residual disease at the

primary site, those who received TBI had a decreased rate of local failure compared with those who received 10 Gy to the primary site alone (22 vs 52%; p = 0.022) [48–50]. Retrospective analysis of the German NB97 experience also supports the role of EBRT to residual disease at the primary tumor site in children with high-risk neuroblastoma. Although numbers of patients were small, the 3-year EFS for children over 1 year of age with international neuroblastoma staging system stage 4 disease who received radiation doses of 36–40 Gy to the site of residual tumor (n = 13) was significantly different from that of patients who did not receive EBRT (n = 23) to residual disease at the primary site (85 vs 25%; p = 0.01) [51]. These data are in keeping with decreased risk of local failure reported by other groups [48–50]. Currently, COG and SIOPEN protocols include a total prescription dose of 21 Gy following gross total resection of the primary tumor. The COG ANBL0532 protocol required a boost of 15 Gy to gross residual disease; results from this trial are currently pending.

Most centers in developed nations use x-ray therapy for delivery of the radiation that is planned using three-dimensional imaging (3D-CRT). In a recent publication from SIOPEN, 99 out of 100 patients treated on a recent study received radiation using 3D-CRT techniques. This technique of delivering x-ray therapy does not allow conformality to protect vital organs. As a result, only 48% of patients on the study were treated according to protocol; the remainder had deviations in delivery to the target due to efforts to protect normal tissues [52]. To improve dosimetry to target volumes and protect normal organs, several groups have investigated the use of intensity-modulated x-ray therapy, intensity modulated arc therapy using x-rays, intraoperative x-ray therapy and proton therapy [53–58]. Each of these modalities has potential to decrease dose to organs at risk, namely liver and kidneys, and excellent local control has been demonstrated with each. However, there are drawbacks to each approach. Intensity modulated x-ray therapy may increase the integral radiation dose by creating a low dose radiation bath. Proton therapy and intraoperative x-ray therapy are relatively new modalities that are available at a limited number of centers. No randomized trials have compared the various approaches to delivery of EBRT to the primary tumor bed, and the design and implementation of such trials is impeded by small patient numbers and differences in access across institutions.

EBRT is also typically employed in high-risk neuroblastoma in an attempt to control sites of disease that appear resistant to induction chemotherapy. Modern COG protocols require the delivery of 21 Gy to all sites of metastatic disease (up to five total) that demonstrate residual abnormal uptake of MIBG on postinduction staging studies. The true benefit of this approach is not well understood, as it has not been studied systematically, but is pursued due to recognitionof the radiosensitivity of neuroblastoma cells. Kushner and colleagues investigated the use of 21 Gy delivered to patients with refractory cranial disease and found that most patients (13/19) had a major response to radiation, with control of cranial disease exceeding control of disease elsewhere (52 vs 33%); the same group has demonstrated a technique for brain sparing when large portions of the skull require radiation [59,60]. Although TBI is no longer utilized in neuroblastoma, as outlined above, improved outcomes for patients who received TBI also supports the use of EBRT to target areas of refractory disease [12]. Future directions may focus on optimal balance of use of therapeutic I-^{131} MIBG versus EBRT for metastatic sites that persist after induction chemotherapy.

■ Recent clinical trial results: postconsolidation therapy

The goal of postconsolidation therapy is to eradicate minimal residual disease. The first agent to be studied for this purpose was isotretinoin. When neuroblastoma cells are exposed to isotretinoin *in vitro*, they exhibit decreased proliferation and morphologic differentiation. Growth arrest and differentiation in response to isotretinoin have been observed in neuroblastoma cell lines initiated from tumors at the time of progression after chemotherapy, suggesting that resistance to cytotoxic chemotherapy does not induce resistance to isotretinoin [61–63]. The effect of isotretinoin was evaluated in the multicenter setting in a randomized controlled trial (CCG3891). Isotretinoin was given twice daily for 2 weeks every 28 days for a total of 6 months. Patients randomized to receive isotretinoin had a decreased risk of tumor recurrence regardless of prior treatment with myeloablative or conventional chemotherapy [12,13]. This study established the role of a differentiating agent as a component of therapy in the minimal residual disease setting.

Immune based therapy, predominantly in the form of antibody therapy, has been explored for the treatment of neuroblastoma for over two decades. The target of immune therapy to date has been GD2, a disialoganglioside antigen that is expressed on tumors of neuroectodermal origin, including neuroblastoma and melanoma. These tumors express GD2 with relatively little heterogeneity among cells [64]. In normal tissues, GD2 expression is largely limited to neurons, melanocytes and peripheral pain fibers, making it a reasonable target for antitumor therapy [65].

MSKCC investigators have extensively studied the murine IgG3 monoclonal antibody specific for GD2 known as 3F8. Results of a Phase I trial demonstrated

that 3F8 could be administered safely despite acute toxicities including pain, hypertension and urticaria [66]. No long-term side effects of immunotherapy were detected [66]. The Phase I trial was followed by a Phase II study of 3F8 alone in children with stage 4 neuroblastoma, and more recently by a Phase II trial of 3F8 combined with GM-CSF. In the latter study, complete resolution of bone marrow disease was demonstrated in 12 of 15 patients [67].

The chimeric monoclonal anti-GD2 antibody ch14.18 has been studied extensively in the multicenter setting in both Germany (Society for Pediatric Oncology and Hematology) and North America (COG). Initial single agent Phase I trials of ch14.18 in patients with refractory neuroblastoma and osteosarcoma demonstrated that acute toxicities were similar to those of 3F8, and included pain, tachycardia, hypertension, fever and urticaria [68]. Chimeric 14.18 was studied in Cooperative German Neuroblastoma trials NB90 and NB97, in which patients received induction treatment and radiotherapy as well as maintenance chemotherapy or myeloablative high-dose chemotherapy followed by ch14.18 or no immunotherapy. No significant difference in 3-year EFS was observed for patients who received ch14.18 versus those who did not (46 vs 44%; p = 0.314), though 3-year OS was higher for patients receiving antibody (68 vs 57%; p = 0.018) [69]. Importantly, with longer follow up, the difference in OS for patients receiving ch14.18 versus no antibody therapy remained statistically significant (46 vs 34%; p = 0.019) [70].

GM-CSF was added to ch14.18 therapy in an effort to enhance antibody dependent cytotoxicity in a Children's Cancer Group trial [71], and IL-2 was subsequently added to the immunotherapy regimen [72]. The combination of ch14.18, GM-CSF and IL-2 with isotretinoin in the postconsolidation setting was further studied in a randomized Phase III trial in the COG (ANBL0032). Patients enrolled on this trial were randomized to receive either isotretinoin alone or isotretinoin and immunotherapy. The trial was stopped early as survival rates for patients receiving immunotherapy were found to be significantly higher than those for patients who received isotretinoin alone. The 2-year EFS from the time of postconsolidation randomization was 66% for patients assigned to receive ch14.18 and cytokines versus 46% for patients randomized to isotretinoin (p = 0.01). Differences in 2-year OS were also significant (86 vs 75%; p = 0.02) [73]. Based on these results, immunotherapy including ch14.18 with GM-CSF and IL-2 has become the standard of care for children with high-risk neuroblastoma in North America. In Europe, an ongoing study will determine the role of IL-2 as a component of immunotherapy, as patients are being randomized to receive ch14.18 alone or ch14.18 in combination with IL-2. Acute toxicities associated with GD-2 directed therapy are not inconsequential, and a significant number of patients experience severe neuropathic pain, fever, capillary leak syndrome and hypersensitivity reactions. Studies designed to determine which patients might be at higher risk for severe toxicities are in progress, and investigators are working to identify groups of patients that may benefit the most from this therapy. Furthermore, newer approaches to immunotherapy continue to be studied, including a humanized form of ch14.18 fused to IL-2 and a mutated ch14.18 antibody [74,75].

Novel therapies: ALK inhibition

ALK is an orphan receptor tyrosine kinase first identified as part of the t(2;5) chromosomal translocation associated with most anaplastic large cell lymphomas and a subset of T-cell non-Hodgkin's lymphomas [76]. In addition to its role in anaplastic large cell lymphomas, ALK signaling is activated in other cancers through *ALK* gene mutations or amplification [76]. In neuroblastoma, mutations in *ALK* are the major cause of hereditary neuroblastoma but can also be somatically acquired in a larger percentage (8–10%) of sporadic cases [77–80]. In addition, *ALK* is amplified in approximately 4% of high-risk neuroblastoma tumors [76]. In those cases of neuroblastoma in which an *ALK* mutation or amplification is present, inhibition of ALK is an attractive therapeutic option.

Crizotinib is an orally bioavailable small-molecule inhibitor of the ALK receptor tyrosine kinase that has been studied in preclinical models of neuroblastoma. Crizotinib has been shown to be highly effective in inhibiting ALK kinase activity, resulting in inhibition of tumor growth [79]. Crizotinib was studied as a single agent in a Phase I clinical trial for children with refractory or relapsed solid tumors or anaplastic large cell lymphoma. The maximum tolerated dose of the drug was found to be 280 mg/m^2/day [81]. Dose-limiting toxicities associated with crizotinib included neutropenia and liver enzyme elevation. Among the 11 patients enrolled on the trial whose neuroblastoma tumors harbored an *ALK* mutation, there was one complete response [81]. A Phase II trial to further investigate the efficacy of this agent in patients whose tumors have been shown to have either *ALK* mutations or amplification of this gene is nearing completion. A Phase I trial designed to assess the toxicity profile of crizotinib in combination with conventional chemotherapy is ongoing. The results of this study will inform plans to incorporate crizotinib into upfront therapy for the subgroup of patients with high-risk neuroblastoma whose tumors have *ALK* aberrations.

Future perspective

Over the past decades, significant advances have been made in understanding the biology of neuroblastoma and determining which patients are at increased risk for relapse. The addition of high-dose chemotherapy, ASCT, isotretinoin and immunotherapy to standard regimens for treatment of patients with high-risk neuroblastoma has improved outcomes. Challenges remain, however, as nearly half of all newly diagnosed patients with high-risk disease will still experience a relapse [1].

The integration of targeted therapies, such as ALK inhibition, is a promising approach for the relatively small percentage of patients whose tumors harbor alterations in this gene. Much work has been done to identify additional tractable therapeutic targets for neuroblastoma therapy using next-generation sequencing techniques. However, several large studies identified a relatively small number of recurrent somatic alterations that represent therapeutic targets [82–84]. The challenge for investigators now is to integrate findings from these studies with work focused on epigenetic changes in tumors, evaluations of host factors, and analysis of emerging data from recent clinical trials as treatment regimens for children with high-risk neuroblastoma continue to evolve.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Background

- Clinical trials throughout the past decade have improved the outcomes for children with high-risk neuroblastoma; however, novel approaches are still needed.

Recent clinical trial results: induction

- Through the use of dose intensive chemotherapy and surgical resection, the goal of induction therapy is to reduce overall tumor burden.

Recent clinical trial results: consolidation

- Consolidation therapy includes autologous stem cell transplant and external beam radiation therapy with future studies evaluating the role of metaiodobenzylguanidine therapy during this phase of treatment.

Recent clinical trial results: postconsolidation therapy

- In order to eradicate minimal residual disease, the differentiating agent isotretinoin is used during postconsolidation therapy.
- Immunotherapy including the antibody Ch14.18 along with cytokines IL-2 and GM-CSF has significantly improved outcomes for children with high-risk neuroblastoma, although toxicity is not negligible.

Novel therapies

- Future studies will likely integrate molecularly targeted therapies into frontline therapy for children whose tumors harbor mutations in genes associated with neuroblastoma oncogenesis.
- Because the number of oncogenic drivers identified to date is relatively small, investigators must now not only evaluate potentially tractable molecular targets for neuroblastoma therapy, but must also evaluate epigenetic changes in tumors, assess the role of host factors in therapy and analyze emerging data from recent clinical trials of existing agents in order to make further improvements in outcomes for children with high-risk neuroblastoma.

References

1 Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. *Lancet* 369(9579), 2106–2120 (2007).

2 Monclair T, Brodeur GM, Ambros PF *et al.* The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. *J. Clin. Oncol.* 27(2), 298–303 (2009).

3 Cecchetto G, Mosseri V, De Bernardi B *et al.* Surgical risk factors in primary surgery for localized neuroblastoma: the LNESG1 study of the European international society of pediatric oncology neuroblastoma group. *J. Clin. Oncol.* 23(33), 8483–8489 (2005).

4 Cohn SL, Pearson AD, London WB *et al.* The international neuroblastoma risk group (INRG) classification system: an INRG task force report. *J. Clin. Oncol.* 27(2), 289–297 (2009).

5 George RE, London WB, Cohn SL *et al.* Hyperdiploidy plus nonamplified MYCN confers a favorable prognosis in children 12 to 18 months old with disseminated neuroblastoma: a pediatric oncology group study. *J. Clin. Oncol.* 23(27), 6466–6473 (2005).

6 Kushner BH, Laquaglia MP, Bonilla MA *et al.* Highly effective induction therapy for stage 4 neuroblastoma in children over 1 year of age. *J. Clin. Oncol.* 12(12), 2607–2613 (1994).

7 Kushner BH, Kramer K, Laquaglia MP, Modak S, Yataghene K, Cheung NK. Reduction from seven to five cycles of intensive induction chemotherapy in children with high-risk neuroblastoma. *J. Clin. Oncol.* 22(24), 4888–4892 (2004).

8 Valteau-Couanet D, Michon J, Boneu A *et al.* Results of induction chemotherapy in children older than 1 year with a stage 4 neuroblastoma treated with the NB 97 french society of pediatric oncology (SFOP) protocol. *J. Clin. Oncol.* 23(3), 532–540 (2005).

9 Park JR, Scott JR, Stewart CF *et al.* Pilot induction regimen incorporating pharmacokinetically guided topotecan for treatment of newly diagnosed high-risk neuroblastoma: a Children's Oncology Group Study. *J. Clin. Oncol.* 29(33), 4351–4357 (2011).

10 Pearson AD, Pinkerton CR, Lewis IJ, Imeson J, Ellershaw C, Machin D. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. *Lancet Oncol.* 9(3), 247–256 (2008).

11 Ladenstein R, Valteau-Couanet D, Brock P *et al.* Randomized Trial of prophylactic granulocyte colony-stimulating factor during rapid COJEC induction in pediatric patients with high-risk neuroblastoma: the European HR-NBL1/SIOPEN study. *J. Clin. Oncol.* 28(21), 3516–3524 (2010).

12 Matthay KK, Reynolds CP, Seeger RC *et al.* Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-*cis*-retinoic acid: a Children's Oncology Group study. *J. Clin. Oncol.* 27(7), 1007–1013 (2009).

13 Matthay KK, Villablanca JG, Seeger RC *et al.* Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-*cis*-retinoic acid. Children's Cancer Group. *N. Engl. J. Med.* 341(16), 1165–1173 (1999).

14 Matthay KK, Reynolds CP, Seeger RC *et al.* Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-*cis*-retinoic acid: a Children's Oncology Group study. *J. Clin. Oncol.* 27(7), 1007–1013 (2009).

15 Berthold F, Boos J, Burdach S *et al.* Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. *Lancet Oncol.* 6(9), 649–658 (2005).

16 Pritchard J, Cotterill SJ, Germond SM, Imeson J, De Kraker J, Jones DR. High dose melphalan in the treatment of advanced neuroblastoma: results of a randomised trial (ENSG-1) by the European Neuroblastoma Study Group. *Pediatr. Blood Cancer* 44(4), 348–357 (2005).

17 Flandin I, Hartmann O, Michon J *et al.* Impact of TBI on late effects in children treated by megatherapy for stage IV neuroblastoma. A study of the French Society of Pediatric Oncology. *Int. J. Radiat. Oncol. Biol. Phys.* 64(5), 1424–1431 (2006).

18 Ladenstein R, Potschger U, Hartman O *et al.* 28 years of high-dose therapy and SCT for neuroblastoma in Europe: lessons from more than 4000 procedures. *Bone Marrow Transplant.* 41(Suppl. 2), S118–S127 (2008).

19 Villablanca JG, Matthay KK, Ramsay NK *et al.* Carboplatin, etoposide, melphalan and local irradiation with autologous bone marrow transplantation for high risk neuroblastoma. *Proc. Am. Soc. Clin. Oncol.* 14, 440 (1995).

20 Kreissman SG, Villablanca JG, Seeger RC *et al.* A randomized Phase III trial of myeloablative autologous peripheral blood stem cell (PBSC) transplant (ASCT) for high-risk neuroblastoma (HR-NB) employing immunomagnetic purged versus unpurged PBSC: a Children's OncologyGroup Study. *J. Clin. Oncol.* 26 (15 Suppl.), Abstract 10011 (2008).

21 Ladenstein RL, Poetschger U, Luksch R *et al.* Busulphan-melphalan as a myeloablative therapy (MAT) for high-risk neuroblastoma: results from the HR-NBL1/SIOPEN trial. *J. Clin. Oncol.* 29(18 Suppl.) 2, (2011).

22 Philip T, Ladenstein R, Zucker JM *et al.* Double megatherapy and autologous bone marrow transplantation for advanced neuroblastoma: the LMCE2 study. *Br. J. Cancer* 67(1), 119–127 (1993).

23 Grupp SA, Stern JW, Bunin N *et al.* Rapid-sequence tandem transplant for children with high-risk neuroblastoma. *Med. Pediatr. Oncol.* 35(6), 696–700 (2000).

24 Kletzel M, Katzenstein HM, Haut PR *et al.* Treatment of high-risk neuroblastoma with triple-tandem high-dose therapy and stem-cell rescue: results of the Chicago Pilot II study. *J. Clin. Oncol.* 20(9), 2284–2292 (2002).

25 Monnereau-Laborde S, Munzer C, Valteau-Couanet D *et al.* A dose-intensive approach (NB96) for induction therapy utilizing sequential high-dose chemotherapy and stem cell rescue in high-risk neuroblastoma in children over 1 year of age. *Pediatr. Blood Cancer* 57(6), 965–971 (2011).

26 Granger M, Grupp SA, Kletzel M *et al.* Feasibility of a tandem autologous peripheral blood stem cell transplant regimen for high risk neuroblastoma in a cooperative group setting: a Pediatric Oncology Group Study: a report from the Children's Oncology Group. *Pediatr. Blood Cancer* 59(5), 902–907 (2012).

27 Sung KW, Son MH, Lee SH *et al.* Tandem high-dose chemotherapy and autologous stem cell transplantation in patients with high-risk neuroblastoma: results of SMC NB-2004 study. *Bone Marrow Transplant.* 48(1), 68–73 (2013).

28 Qayed M, Chiang KY, Ricketts R *et al.* Tandem stem cell rescue as consolidation therapy for high-risk neuroblastoma. *Pediatr. Blood Cancer* 58(3), 448–452 (2012).

29 Saarinen-Pihkala UM, Hovi L, Koivusalo A *et al.* Thiotepa and melphalan based single, tandem, and triple high dose therapy and autologous stem cell transplantation for high risk neuroblastoma. *Pediatr. Blood Cancer* 59(7), 1190–1197 (2012).

30 Seif AE, Naranjo A, Baker DL *et al.* A pilot study of tandem high-dose chemotherapy with stem cell rescue as consolidation for high-risk neuroblastoma: Children's Oncology Group study ANBL00P1. *Bone Marrow Transplant.* 48(7), 947–952 (2013).

31 George RE, Li S, Medeiros-Nancarrow C *et al.* High-risk neuroblastoma treated with tandem autologous peripheral-blood stem cell-supported transplantation: long-term survival update. *J. Clin. Oncol.* 24(18), 2891–2896 (2006).

32 Takaue Y, Watanabe T, Kawano Y *et al.* Isolation and storage of peripheral blood hematopoietic stem cells for autotransplantation into children with cancer. *Blood* 74(4), 1245–1251 (1989).

33 Lasky LC, Fox SB, Smith J, Bostrom B. Collection and use of peripheral blood stem cells in very small children. *Bone Marrow Transplant.* 7(4), 281–284 (1991).

34 Fukuda M, Kojima S, Matsumoto K, Matsuyama T. Autotransplantation of peripheral blood stem cells mobilized by chemotherapy and recombinant human granulocyte colony-stimulating factor in childhood neuroblastoma and non-Hodgkin's lymphoma. *Br. J. Haematol.* 80(3), 327–331 (1992).

35 Klingebiel T, Handgretinger R, Herter M *et al.* Autologous transplantation with peripheral blood stem cells in children and young adults after myeloablative treatment: nonrandomized comparison between GM-CSF and G-CSF for mobilization. *J. Hematother.* 4(4), 307–314 (1995).

36 Takaue Y, Kawano Y, Abe T *et al.* Collection and transplantation of peripheral blood stem cells in very small children weighting 20 kg or less. *Blood* 86(1), 372–380 (1995).

37 Grupp SA, Cohn SL, Wall D, Reynolds CP. Collection, storage, and infusion of stem cells in children with high-risk neuroblastoma: saving for a rainy day. *Pediatr. Blood Cancer* 46(7), 719–722 (2006).

38 Worel N, Apperley JF, Basak GW *et al.* European data on stem cell mobilization with plerixafor in patients with nonhematologic diseases: an analysis of the European consortium of stem cell mobilization. *Transfusion* 52(11), 2395–2400 (2012).

39 Modak S, Cheung IY, Kushner BH, Kramer K, Reich L, Cheung NK. Plerixafor plus granulocyte-colony stimulating factor for autologous hematopoietic stem cell mobilization in patients with metastatic neuroblastoma. *Pediatr. Blood Cancer* 58(3), 469–471 (2012).

40 Donovan J, Temel J, Zuckerman A *et al.* CD34 selection as a stem cell purging strategy for neuroblastoma: preclinical and clinical studies. *Med. Pediatr. Oncol.* 35(6), 677–682 (2000).

41 Powell JL, Bunin NJ, Callahan C, Aplenc R, Griffin G, Grupp SA. An unexpectedly high incidence of Epstein-Barr virus lymphoproliferative disease after CD34+selected autologous peripheral blood stem cell transplant in neuroblastoma. *Bone Marrow Transplant.* 33(6), 651–657 (2004).

42 Reynolds CP, Seeger RC, Vo DD, Black AT, Wells J, Ugelstad J. Model system for removing neuroblastoma cells from bone marrow using monoclonal antibodies and magnetic immunobeads. *Cancer Res.* 46(11), 5882–5886 (1986).

43 Matthay KK, Yanik G, Messina J *et al.* Phase II study on the effect of disease sites, age, and prior therapy on response to iodine-131-metaiodobenzylguanidine therapy in refractory neuroblastoma. *J. Clin. Oncol.* 25(9), 1054–1060 (2007).

44 De Kraker J, Hoefnagel KA, Verschuur AC, Van Eck B, Van Santen HM, Caron HN. Iodine-131-metaiodobenzylguanidine as initial induction therapy in stage 4 neuroblastoma patients over 1 year of age. *Eur. J. Cancer* 44(4), 551–556 (2008).

45 Matthay KK, Tan JC, Villablanca JG *et al.* Phase I dose escalation of iodine-131-metaiodobenzylguanidine with myeloablative chemotherapy and autologous stem-cell transplantation in refractory neuroblastoma: a new approaches to Neuroblastoma Therapy Consortium Study. *J. Clin. Oncol.* 24(3), 500–506 (2006).

46 French S, Dubois SG, Horn B *et al.* ^{131}I-MIBG followed by consolidation with busulfan, melphalan and autologous stem cell transplantation for refractory neuroblastoma. *Pediatr. Blood Cancer* 60(5), 879–884 (2013).

47 Pai Panandiker AS, Mcgregor L, Krasin MJ, Wu S, Xiong X, Merchant TE. Locoregional tumor progression after radiation therapy influences overall survival in pediatric patients with neuroblastoma. *Int. J. Radiat. Oncol. Biol. Phys.* 76(4), 1161–1165 (2010).

48 Haas-Kogan DA, Swift PS, Selch M *et al.* Impact of radiotherapy for high-risk neuroblastoma: a children's cancer group study. *Int. J. Radiat. Oncol. Biol. Phys.* 56(1), 28–39 (2003).

49 Wolden SL, Gollamudi SV, Kushner BH *et al.* Local control with multimodality therapy for stage 4 neuroblastoma. *Int. J. Radiat. Oncol. Biol. Phys.* 46(4), 969–974 (2000).

50 Gatcombe HG, Marcus RB, Katzenstein HM, Tighiouart M, Esiashvili N. Excellent local control from radiation therapy for high-risk neuroblastoma. *Int. J. Radiat. Oncol. Biol. Phys.* 74(5), 1549–1554 (2009).

51 Simon T, Hero B, Bongartz R, Schmidt M, Muller RP, Berthold F. Intensified external-beam radiation therapy improves the outcome of stage 4 neuroblastoma in children >1 year with residual local disease. *Strahlenther. Onkol.* 182(7), 389–394 (2006).

52 Gaze MN, Boterberg T, Dieckmann K *et al.* Results of a quality assurance review of external beam radiation therapy in the international society of paediatric oncology (Europe) neuroblastoma group's high-risk neuroblastoma trial: a SIOPEN study. *Int. J. Radiat. Oncol. Biol. Phys.* 85(1), 170–174 (2013).

53 Pai Panandiker AS, Beltran C, Billups CA, Mcgregor LM, Furman WL, Davidoff AM. Intensity modulated radiation therapy provides excellent local control in high-risk abdominal neuroblastoma. *Pediatr. Blood Cancer* 60(5), 761–765 (2013).

54 Gains JE, Stacey C, Rosenberg I *et al.* Intensity-modulated arc therapy to improve radiation dose delivery in the treatment of abdominal neuroblastoma. *Future Oncol.* 9(3), 439–449 (2013).

55 Mesbah L, Matute R, Usychkin S *et al.* Helical tomotherapy in the treatment of pediatric malignancies: a preliminary report of feasibility and acute toxicity. *Radiat. Oncol.* 6, 102 (2011).

56 Rich BS, Mcevoy MP, Laquaglia MP, Wolden SL. Local control, survival, and operative morbidity and mortality after re-resection, and intraoperative radiation therapy for recurrent or persistent primary high-risk neuroblastoma. *J. Pediatr. Surg.* 46(1), 97–102 (2011).

57 Hattangadi JA, Rombi B, Yock TI *et al.* Proton radiotherapy for high-risk pediatric neuroblastoma: early outcomes and dose comparison. *Int. J. Radiat. Oncol. Biol. Phys.* 83(3), 1015–1022 (2012).

58 Hill-Kayser C, Tochner Z, Both S *et al.* Proton versus photon radiation therapy for patients with high-risk neuroblastoma: the need for a customized approach. *Pediatr. Blood Cancer* 60(10), 1606–1611 (2013).

59 Kushner BH, Cheung NK, Barker CA *et al.* Hyperfractionated low-dose (21 Gy) radiotherapy for cranial skeletal metastases in patients with high-risk neuroblastoma. *Int. J. Radiat. Oncol. Biol. Phys.* 75(4), 1181–1186 (2009).

60 Wolden SL, Barker CA, Kushner BH *et al.* Brain-sparing radiotherapy for neuroblastoma skull metastases. *Pediatr. Blood Cancer* 50(6), 1163–1168 (2008).

61 Sidell N, Altman A, Haussler MR, Seeger RC. Effects of retinoic acid (RA) on the growth and phenotypic expression of several human neuroblastoma cell lines. *Exp. Cell Res.* 148(1), 21–30 (1983).

62 Reynolds CP, Kane DJ, Einhorn PA *et al.* Response of neuroblastoma to retinoic acid *in vitro* and *in vivo*. *Prog. Clin. Biol. Res.* 366, 203–211 (1991).

63 Thiele CJ, Reynolds CP, Israel MA. Decreased expression of N-myc precedes retinoic acid-induced morphological differentiation of human neuroblastoma. *Nature* 313(6001), 404–406 (1985).

64 Schulz G, Cheresh DA, Varki NM, Yu A, Staffileno LK, Reisfeld RA. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. *Cancer Res.* 44(12 Part 1), 5914–5920 (1984).

65 Svennerholm L, Bostrom K, Fredman P *et al.* Gangliosides and allied glycosphingolipids in human peripheral nerve and spinal cord. *Biochim. Biophys. Acta* 1214(2), 115–123 (1994).

66 Cheung NK, Lazarus H, Miraldi FD *et al.* Ganglioside GD2 specific monoclonal antibody 3F8: a phase I study in patients with neuroblastoma and malignant melanoma. *J. Clin. Oncol.* 5(9), 1430–1440 (1987).

67 Cheung NK, Cheung IY, Kushner BH *et al.* Murine anti-GD2 monoclonal antibody 3F8 combined with granulocyte-macrophage colony-stimulating factor and 13-*cis*-retinoic acid in high-risk patients with stage 4 neuroblastoma in first remission. *J. Clin. Oncol.* 30(26), 3264–3270 (2012).

68 Yu AL, Uttenreuther-Fischer MM, Huang CS *et al.* Phase I trial of a human-mouse chimeric anti-disialoganglioside monoclonal antibody ch14.18 in patients with refractory neuroblastoma and osteosarcoma. *J. Clin. Oncol.* 16(6), 2169–2180 (1998).

69 Simon T, Hero B, Faldum A *et al.* Consolidation treatment with chimeric anti-GD2-antibody ch14.18 in children older than 1 year with metastatic neuroblastoma. *J. Clin. Oncol.* 22(17), 3549–3557 (2004).

70 Simon T. Long term outcome of high-risk neuroblastoma patients after immunotherapy with antibody ch14.18 or oral metronomic chemotherapy. *BMC Cancer* 11, 21 (2011).

71 Ozkaynak MF, Sondel PM, Krailo MD *et al.* Phase I study of chimeric human/murine anti-ganglioside G(D2) monoclonal antibody (ch14.18) with granulocyte-macrophage colony-stimulating factor in children with neuroblastoma immediately after hematopoietic stem-cell transplantation: a Children's Cancer Group Study. *J. Clin. Oncol.* 18(24), 4077–4085 (2000).

72 Gilman AL, Ozkaynak MF, Matthay KK *et al.* Phase I study of ch14.18 with granulocyte-macrophage colony-stimulating factor and interleukin-2 in children with neuroblastoma after autologous bone marrow transplantation or stem-cell rescue: a report from the Children's Oncology Group. *J. Clin. Oncol.* 27(1), 85–91 (2009).

73 Yu AL, Gilman AL, Ozkaynak MF *et al.* Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N. Engl. J. Med.* 363(14), 1324–1334 (2010).

74 Shusterman S, London WB, Gillies SD *et al.* Antitumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) Phase II study. *J. Clin. Oncol.* 28(33), 4969–4975 (2010).

75 Sorkin LS, Otto M, Baldwin WM 3rd *et al.* Anti-GD(2) with an FC point mutation reduces complement fixation and decreases antibody-induced allodynia. *Pain* 149(1), 135–142 (2010).

76 Mosse YP, Wood A, Maris JM. Inhibition of ALK signaling for cancer therapy. *Clin. Cancer Res.* 15(18), 5609–5614 (2009).

77 Mosse YP, Laudenslager M, Longo L *et al.* Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* 455(7215), 930–935 (2008).

78 Janoueix-Lerosey I, Lequin D, Brugieres L *et al.* Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature* 455(7215), 967–970 (2008).

79 George RE, Sanda T, Hanna M *et al.* Activating mutations in ALK provide a therapeutic target in neuroblastoma. *Nature* 455(7215), 975–978 (2008).

80 Chen Y, Takita J, Choi YL *et al.* Oncogenic mutations of ALK kinase in neuroblastoma. *Nature* 455(7215), 971–974 (2008).

81 Mosse YP, Lim MS, Voss SD *et al.* Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group Phase I consortium study. *Lancet Oncol.* 14(6), 472–480 (2013).

82 Sausen M, Leary RJ, Jones S *et al.* Integrated genomic analyses identify ARID1A and ARID1B alterations in the childhood cancer neuroblastoma. *Nat. Genet.* 45(1), 12–17 (2013).

83 Pugh TJ, Morozova O, Attiyeh EF *et al.* The genetic landscape of high-risk neuroblastoma. *Nat. Genet.* 45(3), 279–284 (2013).

84 Molenaar JJ, Koster J, Zwijnenburg DA *et al.* Sequencing of neuroblastoma identifies chromothripsis and defects in neuritogenesis genes. *Nature* 483(7391), 589–593 (2012).