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New Aspects of Neuroblastoma Treatment: ASPHO 2011 Symposium Review

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Abstract

Neuroblastoma is the most common extracranial solid tumor of childhood, and the outcomes for children with high-risk and relapsed disease remain poor. However, new international strategies for risk stratification and for treatment based on novel tumor targets and including immunotherapy are being employed in attempts to improve the outcomes of children with neuroblastoma. A new international neuroblastoma risk classification system has been developed which is being incorporated into cooperative group clinical trials in North America, Japan, and Europe, resulting in standardized approaches for the initial evaluation and treatment stratification of neuroblastoma patients. Furthermore, novel treatment regimens are being developed based on improved understanding of neuroblastoma biology and on the recruitment of the immune system to specifically target neuroblastoma tumors. These approaches will lead to new therapeutic strategies that likely will improve the outcomes for children with neuroblastoma worldwide.

Keywords

immunotherapy; INRG; neuroblastoma

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor in childhood and accounts for approximately 8% of childhood cancer. Approximately 40% of neuroblastoma tumors are classified as high-risk using current risk stratification criteria. Treatment for children with high-risk neuroblastoma includes combinations of chemotherapy, autologous stem cell transplantation, surgery, and radiation therapy. However, while children with low- or

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intermediate-risk neuroblastoma have survival rates over 90%, children with high-risk neuroblastoma have very poor outcomes despite this aggressive treatment, with significant short- and long-term complications [1]. Cases of high-risk neuroblastoma are also associated with frequent relapses and treatment-resistant tumors. Children with recurrent or refractory neuroblastoma have a less than 50% response rate to alternative regimens and a very poor 5-year overall survival rate [2,3]. Further increases in therapeutic dose intensity will be associated with prohibitive short-term and long-term toxicities, and novel treatment strategies are required for children with high-risk and recurrent neuroblastoma.

A new international neuroblastoma risk classification system (the International Neuroblastoma Risk Group (INRG)) has been developed using statistically significant, clinically relevant factors that were identified after review of data from thousands of international neuroblastoma patients. The use of this new risk classification system will result in significant changes in the initial evaluation and subsequent treatment of neuroblastoma patients and in a more consistent approach to neuroblastoma risk stratification. While treatment of relapsed and refractory neuroblastoma remains a challenge, novel treatments are currently being developed based on a better understanding of neuroblastoma biology and on techniques to harness and adapt the immune system to specifically target neuroblastoma tumors. These approaches will dramatically increase the options available for treatment of children with neuroblastoma and potentially improve the outcomes of children with neuroblastoma worldwide.

This review will summarize the presentations given at the “New Aspects of Neuroblastoma Treatment” symposium at the 2011 American Society of Pediatric Hematology/Oncology annual meeting in Baltimore.

THE INTERNATIONAL NEUROBLASTOMA RISK GROUP (INRG) CLASSIFICATION SYSTEM: PAST, PRESENT, AND FUTURE

Neuroblastoma tumors are characterized by diverse clinical behavior, reflecting their biologic heterogeneity. Treatment strategies for children with neuroblastoma have been tailored according to the predicted response to therapy and risk of relapse for more than 40 years [1], and treatment stratification has become increasingly important as we obtain a better understanding of clinical and biological risk factors. The development and use of international staging systems (the International Neuroblastoma Staging System (INSS)) has provided consistency in the staging of patients with neuroblastoma worldwide [4,5]. However, cooperative groups from different regions of the world have not consistently used the same markers to classify patient risk, and therefore the patient cohorts treated on risk-based studies are not uniform. For example, while the presence or absence of *MYCN* gene amplification is a universal factor for risk-group stratification, other prognostically significant genetic aberrations and tumor biologic features have not been routinely incorporated into risk classification schemas. These disparities have made it difficult to compare the results of clinical trials performed throughout the world. To address this concern, in 2004, investigators and members from major national and international cooperative groups, including the Children’s Oncology Group (COG), the German Gesellschaft für Pädiatrische Onkologie und Hämatologie (GPOH), the Japanese Advanced

Neuroblastoma Study Group (JANB), the Japanese Infantile Neuroblastoma Co-operative Study Group (JINCS), and the International Society of Paediatric Oncology Europe Neuroblastoma Group (SIOPEN), were invited to participate in an effort to develop a novel classification system, based on evaluations of available clinical and biological data. As a result of this collaborative effort, the International Neuroblastoma Risk Group (INRG) classification system was created [6].

The INRG classification system was developed based on analysis of the data from over 8,800 patients with neuroblastoma assembled from these combined datasets. Thirteen clinical and biological variables were analyzed for effects on event-free survival in this large patient cohort, including patient age at diagnosis, tumor stage, serum lactate dehydrogenase and ferritin levels, tumor histological category, grade of differentiation, tumor mitosis-karyorrhexis index (MKI), *MYCN* gene amplification status, the presence or absence of chromosome 1p or 11q abnormalities, DNA ploidy, the primary tumor site (adrenal or nonadrenal) and the presence or absence of metastatic disease. A survival tree methodology was employed, using analyses of each variable at each branch point, with branches created by dividing the cohort into two subgroups at each point using the most significant identified variable.

Seven clinically relevant and statistically significant factors (tumor stage, patient age, tumor histological category and grade of differentiation, *MYCN* gene amplification status, chromosome 11q aberration, and DNA ploidy) were incorporated into the INRG classification system (Table I). Tumor staging in the INRG system is based on a new pre-treatment staging system (the INRG staging system) composed of four stages, two for locoregional disease (L1 and L2), and two for metastatic disease (M and MS). The extent of locoregional disease is evaluated pre-surgically by the absence or presence of anatomic surgical risk factors identified on imaging studies, such as organ invasion or encasement of blood vessels, with tumors having any of these image defined risk factors (IDRFs) classified as stage L2, while all other tumors are classified as L1. Neuroblastoma tumors with disseminated metastases are classified as stage M, analogous to INSS stage 4, with the exception of infants with metastases limited to skin, liver and bone marrow, who are classified as stage MS, analogous to INSS stage 4S [7].

Although a number of genetic aberrations are strongly associated with outcome in neuroblastoma, only genetic factors that were routinely evaluated by the large cooperative groups between 1990 and 2002 were included in the analysis of prognostic criteria for the INRG classification system. New technologies are now available for whole-genome analysis, and numerous studies suggest that this approach will lead to a further refinement of risk stratification. Array-chromosomal comparative genomic hybridization (CGH), which provides information regarding overall genomic instability, has been shown to add critical prognostic information to individual genetic aberrations [8,9]. The prognostic effects of mRNA gene expression profiles on neuroblastoma patient outcomes have also been reported by numerous investigators [10–19]. De Preter and co-workers [20] have built an independently prognostic 42-gene signature based on the reanalysis of gene expression studies using different microarray platforms comprising 582 patients. The power of these analyses is clearly shown by the identification of known neuroblastoma risk factors (such as

the *MYCN* gene) in addition to several consistently identified novel genes and pathways whose role in neuroblastoma pathogenesis remains to be elucidated (Table II). In other preliminary studies, microRNA profiles [21,22] and epigenetic changes [23,24] have also been reported to be associated with survival in patients with neuroblastoma. However, these studies will need to be validated in larger multinational patient cohorts to establish whether these changes are independent of other genetic risk factors.

Clearly, risk classification will continue to be refined with advances in technology and in our understanding of the fundamental genomic alterations that are associated with tumor behavior and patient outcomes. The optimal prognostic classifier is likely to require an integrated analysis that includes profiling of mRNA and microRNA expression, epigenetic modifications, and whole genome copy number variations, which will require the development of technologies that will yield rapid and reproducible results in a cost-effective manner. To remain clinically relevant, the INRG classification system must continue to evolve as new information is integrated into the diagnostic setting and confirmed to be prognostic in prospective studies. With the identification of smaller cohorts of biologically distinct neuroblastoma patients, international collaboration will become increasingly important, and the need for international consensus will become even more critical.

THE ROLE OF IMMUNOTHERAPY FOR NEUROBLASTOMA

With the critical need for new treatment strategies for children with neuroblastoma, the use of immunotherapy has been an attractive option, as targeting tumor-specific antigens should result in both improved efficacy and decreased toxicity. However, the use of immunotherapy for cancer treatment has been limited in the past by mechanisms employed by tumor cells to evade the host immune system, including the down-regulation of major histocompatibility complex (MHC) class I and II molecule expression; the secretion of inhibitory cytokines into the tumor microenvironment; and the recruitment of other suppressive cells (i.e., T regulatory cells) leading to decreased tumor immunogenicity [25]. However, researchers have identified approaches to circumvent these limitations, leading to three immunotherapeutic modalities available for patients with neuroblastoma: monoclonal antibodies, vaccination, and adoptive cellular therapy.

Monoclonal antibodies (MAbs) recognize tumor-specific antigens and recruit effector complexes directly to the tumor cell, resulting in tumor cell death primarily through antibody-dependent cell-mediated cytotoxicity (ADCC). Neuroblastoma tumor associated antigens (TAAs) targeted by MAbs include the gangliosides GD2, GD3, and GM3 and the glycoproteins CD56, L1-CAM, and GP95 [26,27]. 3F8, a murine antibody targeting GD2, was the first MAb used in therapeutic clinical trials for patients with neuroblastoma [28], and treatment with 3F8 has resulted in clinical responses, including sustained complete responses, in children with neuroblastoma, especially in those with low disease burden [29,30].

Treatment with anti-GD2 MAbs is associated with significant side effects, including pain from reaction with GD2 antigens expressed on peripheral nerves and significant hypersensitivity reactions [31,32]. Investigators have therefore focused on ways to decrease

the immunogenicity and the side effects of these MAbs (Table III). Ch14.18, a chimeric anti-GD2 MAb containing both murine and human sequences, was tested in a large phase III randomized clinical trial through the COG. When compared to standard maintenance therapy, the addition of ch14.18, IL-2, and GM-CSF was associated with an improvement in both 2 year event-free and overall survival (46% vs. 66% and 75% vs. 86%, respectively) [33], leading to closure of the study arm with maintenance therapy alone and continued studies to better assess the side effect profile of ch14.18 administration. Additional efforts are also currently underway to further enhance the efficacy and minimize the toxicity of MAb therapy using antibodies with further modifications, including direct antibody linkage to cytokines, linkage of antibodies to radionuclides, and generation of MAb mutants with reduced complement activation [34–36].

In contrast to antibody therapy, developing an effective vaccine for neuroblastoma has been a considerable challenge. Disease heterogeneity and down-regulation of MHC and co-stimulatory molecules by neuroblastoma tumors limit the effectiveness of any tumor-specific T cell immune response induced by a vaccine [32]. In order to increase the presentation of a wide range of TAAs and account for tumor heterogeneity, initial studies of vaccines for children with neuroblastoma employed cellular extracts or whole cell products, with some significant clinical responses seen [37–40]. More recent studies evaluated an allogeneic tumor cell vaccine comprised of a neuroblastoma tumor cell line modified to secrete both IL-2 and lymphotactin, a T cell recruiting chemokine [41]. Clinical responses were seen in 11 of 28 treated patients, with 4 complete responses (two sustained more than 4 years after vaccination), 2 partial responses, and 5 patients with stable disease [32,41,42]. Currently investigators are evaluating whether the addition of a second, unmodified, cell line expressing a distinct set of TAAs, either in the setting of minimal residual disease or in combination with metronomic chemotherapy, will increase the breadth of the resulting immune response and overall anti-tumor response.

The use of adoptive cellular therapies for neuroblastoma has been limited by the technical and regulatory requirements for manufacture and administration of cellular products. Nonetheless, T cells genetically modified to express chimeric antigen receptors (CARs) directed against TAAs have been tested successfully in adult and pediatric cancer patients [43,44]. Initial studies using CAR-expressing T cells targeting CD171 in children with neuroblastoma demonstrated partial responses in one of six patients, with no significant toxicities reported [45]. Subsequent studies employing T cells modified to express CARs directed against GD2 demonstrated clinical responses in 5 of 11 patients, with 3 complete responses that were sustained over 2 years in 2 of the 3 patients [46,47]. The only treatment-related adverse events identified were low-grade fever and mild to moderate local pain at disease sites. Additionally, detection of GD2-targeted T cells beyond 6 weeks was associated with superior clinical outcome, and duration of the modified T cell persistence within the entire cohort was highly concordant with the percentage of $CD4^+$ cells and central memory cells within the infused T cell product [47]. The successes of these and other immunotherapy trials for children with neuroblastoma provide hope for the use of immune therapy as a component of future neuroblastoma treatment regimens.

DISCOVERY AND EXPLOITATION OF MOLECULAR TARGETS IN NEUROBLASTOMA

Despite recent advances, 50–60% of patients with high-risk neuroblastoma will develop recurrent disease, and to date there are no well-established, curative treatment regimens for these patients. Over the past decade, extensive investigations into the biology of neuroblastoma pathogenesis have resulted in a wide range of novel targets for new therapies, and several agents have been identified that are highly active in preclinical models.

Several recent studies have identified a role for anaplastic lymphoma kinase (ALK) in the pathogenesis of familial neuroblastoma [48–51]. ALK was initially characterized as a fusion partner with nucleophosmin in chromosomal translocations present in high-grade lymphomas and has subsequently been identified in translocations in a number of malignancies [52,53]. Full-length ALK is primarily expressed in the nervous system and is involved in neuronal differentiation [54]. Activating mutations of ALK are found in a large majority of familial cases of neuroblastoma, which accounts for approximately 2% of all cases of neuroblastoma, and ALK gene mutations or gene amplifications have been identified in up to 15% of sporadic high-risk neuroblastoma cases [48,55]. Furthermore, wild-type ALK expression is elevated in high-risk compared to low-risk neuroblastoma tumors [56]. Subsequent studies have demonstrated the efficacy of ALK inhibition in preclinical models of neuroblastoma with high expression of wild type or mutant ALK [56,57], and early phase clinical trials utilizing the novel ALK inhibitor PF-02341066 in children with relapsed and refractory solid tumors are underway through the COG.

Other recent studies have identified the Aurora A kinase as a potential therapeutic target in neuroblastoma tumors. The Aurora A kinase has a critical role regulating the mitotic checkpoint complex and is essential for appropriate completion of mitosis [58]. However, when aberrantly over-expressed, Aurora A leads to genomic instability, suppression of p53 function and resistance to apoptosis [59]. Aurora A is highly expressed in many adult tumors, including breast and ovarian cancers [60–62], while in neuroblastoma tumors, expression of Aurora A kinase correlates with advanced stage and high-risk disease [63,64]. Small molecule inhibitors of this kinase block proliferation and soft agar colony formation of neuroblastoma tumor cells and increase sensitivity to chemotherapy [63]. Subsequent studies using the Aurora A kinase inhibitor MLN8237 demonstrated efficacy against preclinical neuroblastoma models [65], and a phase I/II clinical trial of MLN8237 is also underway through the COG.

Other identified targets include the RET tyrosine kinase, which is expressed primarily on neural crest-derived cells and is required for peripheral nervous system maturation. Studies have demonstrated that RET is required for retinoic acid-induced neuroblastoma differentiation [66], and that RET inhibition is effective in neuroblastoma preclinical models [67]. Other recent studies have identified the polo-like kinase 1 (PLK1) as a potential target for neuroblastoma therapy, based on screens of a library of kinase inhibitors in neuroblastoma preclinical models [68], while a screening study using an siRNA library identified the checkpoint kinase 1 (CHK1) as a potential target [69]. Transcriptome analysis

of neuroblastoma tumor formation in the *MYCN* transgenic mouse model identified the centromere-associated protein E (CENPE) as an additional potential therapeutic target [70].

Two large collaborative research efforts have focused on discovering additional targets for neuroblastoma therapy. The Therapeutically Applicable Research to Generate Effective Treatments (TARGET) program, coordinated by the National Cancer Institute Office of Cancer Genomics and the Cancer Therapy Evaluation Program (CTEP), employs genomic profiling and sequencing of neuroblastoma tumors to identify additional new targets and corresponding novel agents likely to target the identified proteins, genes, or pathways. In addition, the collaborators in the Pediatric Preclinical Testing Program (PPTP) employ in vitro and in vivo preclinical models of pediatric cancers to screen novel agents in the early stages of clinical development for activity against pediatric cancers [71]. The complementary TARGET and PPTP approaches are large efforts that, in combination with continued research studies, will hopefully lead to additional treatment options for patients with neuroblastoma.

CONCLUSIONS

Modern treatment for children with neuroblastoma is based on precise prognostication and risk-based treatment strategies. Recent international consensus regarding the criteria to define risk groups has been achieved, and the INRG classification system will greatly facilitate the comparison of risk-based clinical trials conducted in different regions of the world and the development of international collaborative studies. The treatment of patients with high-risk and relapsed neuroblastoma remains a challenge, however. Identification of novel therapies harnessing the innate immune system and of novel agents for treatments targeted at biologically relevant pathways may provide new opportunities for improved outcomes for these patients.

The future holds promise for making considerable advances in our understanding and treatment of neuroblastoma. The critical mutations and defects that cause neuroblastoma or influence its natural history are rapidly being identified, providing the key molecular targets for future rational drug development, and a wide range of novel therapies are currently undergoing preclinical and clinical evaluation. The extensive national and international collaborations currently focused on studying this disease will provide opportunities to test these new approaches in carefully controlled clinical trials that should result in more precise and effective therapeutic regimens. In the meantime, improved international strategies to stratify patients based on established clinical and biological criteria will serve to ensure that patients receive appropriate therapeutic intensity.

Abbreviations

ASPHO	American Society of Pediatric Hematology/Oncology
INRG	International Neuroblastoma Risk Group
IDRF	image-defined risk factor

PPTP	Pediatric Preclinical Testing Program
TARGET	therapeutically applicable research to generate effective treatments
MKI	mitosis-karyorrhexis index
COG	Children's Oncology Group
INSS	International Neuroblastoma Staging System
CGH	comparative genomic hybridization
MHC	major histocompatibility complex
MAbs	monoclonal antibodies
ADCC	antibody dependent cell mediated cytotoxicity
TAA	tumor associated antigen
GM-CSF	granulocyte/monocyte-colony stimulating factor
IL-2	interleukin-2
NK	natural killer
ALK	anaplastic lymphoma kinase
CTEP	cancer therapy evaluation program

References

1. Maris JM, Hogarty MD, Bagatell R, et al. Neuroblastoma. Lancet. 2007; 369:2106–2120. [PubMed: 17586306]
2. Donfrancesco A, Jenkner A, Castellano A, et al. Ifosfamide/carboplatin/etoposide (ICE) as front-line, topotecan/cyclophosphamide as second-line and oral temozolomide as third-line treatment for advanced neuroblastoma over one year of age. Acta Pediatr Suppl. 2004; 445:6–11.
3. London WB, Castel V, Monclair T, et al. Clinical and biological features predictive of survival after relapse of neuroblastoma: A report from the International Neuroblastoma Risk Group Project. J Clin Oncol. 2011; 29:3286–3292. [PubMed: 21768459]
4. Brodeur GM, Seeger RC, Barrett A, et al. International Criteria for diagnosis, staging, and response to treatment in patients with neuroblastoma. J Clin Oncol. 1988; 6:1874–1881. [PubMed: 3199170]
5. Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the International Criteria for Neuroblastoma Diagnosis, Staging, and Response to Treatment. J Clin Oncol. 1993; 11:1466–1477. [PubMed: 8336186]
6. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) Classification System: An INRG task force report. J Clin Oncol. 2009; 27:289–297. [PubMed: 19047291]
7. Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) Staging System: An INRG task force report. J Clin Oncol. 2009; 27:298–303. [PubMed: 19047290]
8. Schleiermacher G, Michon J, Huon I, et al. Chromosomal CGH identifies patients with a higher risk of relapse in neuroblastoma without MYCN amplification. Br J Cancer. 2007; 97:238–246. [PubMed: 17579628]
9. Janoueix-Lerosey I, Schleiermacher G, Michels E, et al. Overall genomic pattern is a predictor of outcome in neuroblastoma. J Clin Oncol. 2009; 27:1026–1033. [PubMed: 19171713]
10. Oberthuer A, Hero B, Berthold F, et al. Prognostic impact of gene expression-based classification for neuroblastoma. J Clin Oncol. 2010; 28:3506–3515. [PubMed: 20567016]

11. Vermeulen J, De Preter K, Naranjo A, et al. Predicting outcomes for children with neuroblastoma using a multigene-expression signature: A retrospective SIOPEN/COG/GPOH study. Lancet Oncol. 2009; 10:663–671. [PubMed: 19515614]
12. Wang Q, Diskin S, Rappaport E, et al. Integrative genomics identifies distinct molecular classes of neuroblastoma and shows that multiple genes are targeted by regional alterations in DNA copy number. Cancer Res. 2006; 66:6050–6062. [PubMed: 16778177]
13. Asgharzadeh S, Pique-Regi R, Sposto R, et al. Prognostic significance of gene expression profiles of metastatic neuroblastomas lacking MYCN gene amplification. J Natl Cancer Inst. 2006; 98:1193–1203. [PubMed: 16954472]
14. Ohira M, Oba S, Nakamura Y, et al. Expression profiling using a tumor-specific cDNA microarray predicts the prognosis of intermediate risk neuroblastomas. Cancer Cell. 2005; 7:337–350. [PubMed: 15837623]
15. Wei JS, Greer BT, Westermann F, et al. Prediction of clinical outcome using gene expression profiling and artificial neural networks for patients with neuroblastoma. Cancer Res. 2004; 64:6883–6891. [PubMed: 15466177]
16. Schramm A, Schulte JH, Klein-Hitpass L, et al. Prediction of clinical outcome and biological characterization of neuroblastoma by expression profiling. Oncogene. 2005; 24:7902–7912. [PubMed: 16103881]
17. Oberthuer A, Berthold F, Warnat P, et al. Customized oligonucleotide microarray gene expression-based classification of neuroblastoma patients outperforms current clinical risk stratification. J Clin Oncol. 2006; 24:5070–5078. [PubMed: 17075126]
18. Schramm A, Vandesompele J, Schulte JH, et al. Translating expression profiling into a clinically feasible test to predict neuroblastoma outcome. Clin Cancer Res. 2007; 13:1459–1465. [PubMed: 17332289]
19. Kamei N, Hiyama K, Yamaoka H, et al. Evaluation of genes identified by microarray analysis in favorable neuroblastoma. Pediatr Surg Int. 2009; 25:931–937. [PubMed: 19701758]
20. De Preter K, Vermeulen J, Brors B, et al. Accurate outcome prediction in neuroblastoma across independent data sets using a multigene signature. Clin Cancer Res. 2010; 16:1532–1541. [PubMed: 20179214]
21. Bray I, Bryan K, Prenter S, et al. Widespread dysregulation of miRNAs by MYCN amplification and chromosomal imbalances in neuroblastoma: Association of miRNA expression with survival. PLoS ONE. 2009; 4:e7850. [PubMed: 19924232]
22. Wei JS, Johansson P, Chen QR, et al. MicroRNA profiling identifies cancer-specific and prognostic signatures in pediatric malignancies. Clin Cancer Res. 2009; 15:5560–5568. [PubMed: 19706822]
23. Abe M, Watanabe N, McDonell N, et al. Identification of genes targeted by CpG island methylator phenotype in neuroblastomas, and their possible integrative involvement in poor prognosis. Oncology. 2008; 74:50–60. [PubMed: 18544995]
24. Yang Q, Kiernan CM, Tian Y, et al. Methylation of CASP8, DCR2, and HIN-1 in neuroblastoma is associated with poor outcome. Clin Cancer Res. 2007; 13:3191–3197. [PubMed: 17545522]
25. Whiteside TL. Immune suppression in cancer: Effects on immune cells, mechanisms and future therapeutic intervention. Semin Cancer Biol. 2006; 16:3–15. [PubMed: 16153857]
26. Johnson E, Dean SM, Sondel PM. Antibody-based immunotherapy in high-risk neuroblastoma. Expert Rev Mol Med. 2007; 9:1–21. [PubMed: 18081947]
27. Cheung, NK.; Sondel, PM. Neuroblastoma immunology and immunotherapy. In: Cohn, S.; Cheung, NK., editors. Neuroblastoma. Berlin, Heidelberg, New York: Springer Press; 2005. p. 223-242.
28. 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. 1987; 5:1430–1440. [PubMed: 3625258]
29. Cheung NK, Kushner BH, Yeh SD, et al. 3F8 monoclonal antibody treatment of patients with stage 4 neuroblastoma: A phase II study. Int J Oncol. 1998; 12:1299–1306. [PubMed: 9592190]
30. Modak S, Cheung NK. Neuroblastoma: Therapeutic strategies for a clinical enigma. Cancer Treat Rev. 2010; 36:307–317. [PubMed: 20227189]

31. Leen AM, Rooney CM, Foster AE. Improving T cell therapy for cancer. Annu Rev Immunol. 2007; 25:243–265. [PubMed: 17129181]
32. Louis CU, Brenner MK. Cellular immunotherapy for neuroblastoma: A Review of current vaccine and adoptive T Cell therapeutics. Curr Pharm Des. 2009; 15:424–429. [PubMed: 19199969]
33. Yu AL, Gilman AL, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med. 2010; 363:1324–1334. [PubMed: 20879881]
34. Carter P. Improving the efficacy of antibody-based cancer therapies. Nat Rev Cancer. 2001; 1:118–129. [PubMed: 11905803]
35. Yang RK, Sondel PM. Anti-GD2 strategy in the treatment of neuroblastoma. Drugs Future. 2010; 35:665. [PubMed: 21037966]
36. Kramer K, Kushner BH, Modak S, et al. Compartmental intrathecal radioimmunotherapy: Results for treatment for metastatic CNS neuroblastoma. J Neurooncol. 2010; 97:409–418. [PubMed: 19890606]
37. Caruso DA, Orme LM, Amor GM, et al. Results of a phase I study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children with stage 4 neuroblastoma. Cancer. 2005; 103:1280–1291. [PubMed: 15693021]
38. Geiger JD, Hutchinson RJ, Hohenkirk LF, et al. Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific t cells and mediate tumor regression. Cancer Res. 2001; 61:8513–8519. [PubMed: 11731436]
39. Bowman L, Grossmann M, Rill D, et al. IL-2 adenovector-transduced autologous tumor cells induce antitumor immune responses in patients with neuroblastoma. Blood. 1998; 92:1941–1949. [PubMed: 9731051]
40. Bowman LC, Grossmann M, Rill D, et al. Interleukin-2 gene-modified allogeneic tumor cells for treatment of relapsed neuroblastoma. Hum Gene Ther. 1998; 9:1303–1311. [PubMed: 9650615]
41. Rousseau RF, Haight AE, Hirschmann-Jax C, et al. Local and systemic effects of an allogeneic tumor cell vaccine combining transgenic human lymphotactin with interleukin-2 in patients with advanced or refractory neuroblastoma. Blood. 2003; 101:1718–1726. [PubMed: 12406881]
42. Rousseau RF, Brenner MK. Vaccine therapies for pediatric malignancies. Cancer J. 2005; 11:331–339. [PubMed: 16197723]
43. Porter DL, Levine BL, Kalos M, et al. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011; 365:725–733. [PubMed: 21830940]
44. Di Stasi A, Tey S-K, Dotti G, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N Engl J Med. 2011; 365:1673–1683. [PubMed: 22047558]
45. Park JR, Digiusto DL, Slovak M, et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Mol Ther. 2007; 15:825–833. [PubMed: 17299405]
46. Pule MA, Savoldo B, Myers GD, et al. Virus-Specific t cells engineered to coexpress tumor-specific receptors: Persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008; 14:1264–1270. [PubMed: 18978797]
47. Louis CU, Savoldo B, Dotti G, et al. Anti-tumor activity and long-term fate of chimeric antigen receptor positive T-cells in patients with neuroblastoma. Blood. 2011; 118:6050–6056. [PubMed: 21984804]
48. Mosse YP, Laudenslager M, Longo L, et al. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature. 2008; 455:930–935. [PubMed: 18724359]
49. Janoueix-Lerosey I, Lequin D, Brugieres L, et al. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. Nature. 2008; 455:967–970. [PubMed: 18923523]
50. Chen Y, Takita J, Choi YL, et al. Oncogenic mutations of ALK kinase in neuroblastoma. Nature. 2008; 455:971–974. [PubMed: 18923524]
51. George RE, Sanda T, Hanna M, et al. Activating mutations in ALK provide a therapeutic target in neuroblastoma. Nature. 2008; 455:975–978. [PubMed: 18923525]
52. Morris SW, Kirstein MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science. 1994; 263:1281–1284. [PubMed: 8122112]

53. Lin E, Li L, Guan Y, et al. Exon array profiling detects EML4-ALK fusion in breast, colorectal, and non-small cell lung cancers. Mol Cancer Res. 2009; 7:1466–1476. [PubMed: 19737969]
54. Iwahara T, Fujimoto J, Wen D, et al. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene. 1997; 14:439–449. [PubMed: 9053841]
55. Caren H, Abel F, Kogner P, et al. High incidence of DNA mutations and gene amplifications of the ALK gene in advanced sporadic neuroblastoma tumors. Biochem J. 2008; 416:153–159. [PubMed: 18990089]
56. Passoni L, Longo L, Collini P, et al. Mutation-independent anaplastic lymphoma kinase overexpression in poor prognosis neuroblastoma patients. Cancer Res. 2009; 69:7338–7346. [PubMed: 19723661]
57. Mosse YP, Wood A, Maris JM. Inhibition of ALK signaling for cancer therapy. Clin Cancer Res. 2009; 15:5609–5614. [PubMed: 19737948]
58. Zhou H, Kuang J, Zhong L, et al. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy, and transformation. Nat Genet. 1998; 20:189–193. [PubMed: 9771714]
59. Sen S, Katayama H, Sasai K. Functional significance of aurora kinase A in centrosome amplification and genomic instability. Adv Exp Med Biol. 2008; 617:99–108. [PubMed: 18497034]
60. Macarulla T, Ramos FJ, Tabernero J. Aurora kinase family: A new target for anticancer drug. Recent Pat Anticancer Drug Discov. 2008; 3:114–122. [PubMed: 18537754]
61. Fu S, Hu W, Kavanagh JJ, et al. Targeting Aurora kinases in ovarian cancer. Expert Opin Ther Targets. 2006; 10:77–85. [PubMed: 16441230]
62. Hu W, Kavanagh JJ, Deaver M, et al. Frequent overexpression of STK15/Aurora-A/BTAK and chromosomal instability in tumorigenic cell cultures derived from human ovarian cancer. Oncol Res. 2005; 15:49–57. [PubMed: 15839305]
63. Shang X, Burlingame SM, Okcu MF, et al. Aurora A is a negative prognostic factor and a new therapeutic target in human neuroblastoma. Mol Cancer Ther. 2009; 8:2461–2469. [PubMed: 19671766]
64. Otto T, Horn S, Brockmann M, et al. Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. Cancer Cell. 2009; 15:67–78. [PubMed: 19111882]
65. Maris JM, Morton CL, Gorlick R, et al. Initial testing of the Aurora kinase A inhibitor MLN8237 by the Pediatric Preclinical Testing Program (PPTP). Pediatr Blood Cancer. 2010; 55:26–34. [PubMed: 20108338]
66. Oppenheimer O, Cheung N-K, Gerald WL. The RET oncogene is a critical component of transcriptional programs associated with retinoic acid-induced differentiation in neuroblastoma. Mol Cancer Ther. 2007; 6:1300–1309. [PubMed: 17431108]
67. Zage PE, Zeng L, Palla S, et al. A novel therapeutic combination for neuroblastoma: The VEGF/EGF/RET inhibitor vandetanib with 13-cis-retinoic acid. Cancer. 2010; 116:2465–2475. [PubMed: 20225331]
68. Grinshtein N, Datti A, Fujitani N, et al. Small molecule kinase inhibitor screen identifies polo-like kinase 1 as a target for neuroblastoma tumor-initiating cells. Cancer Res. 2011; 71:1385–1395. [PubMed: 21303981]
69. Cole KA, Huggins J, Laquaglia M, et al. RNAi screen of the protein kinome identifies checkpoint kinase 1 (CHK1) as a therapeutic target in neuroblastoma. Proc Natl Acad Sci USA. 2011; 108:3336–3341. [PubMed: 21289283]
70. Balamuth NJ, Wood A, Wang Q, et al. Serial transcriptome analysis and cross-species integration identifies *centromere-associated protein E* as a novel neuroblastoma target. Cancer Res. 2010; 70:2749–2758. [PubMed: 20233875]
71. Houghton PJ, Morton CL, Tucker C, et al. The Pediatric Preclinical Testing Program: Description of models and early testing results. Pediatr Blood Cancer. 2007; 49:928–940. [PubMed: 17066459]
72. Cheung NK, Burch L, Kushner BH, et al. Monoclonal antibody 3F8 can effect durable remissions in neuroblastoma patients refractory to chemotherapy: A phase II trial. Prog Clin Biol Res. 1991; 366:395–400. [PubMed: 2068154]

73. Cheung NK, Kushner BH, Yeh SJ, et al. 3F8 monoclonal antibody treatment of patients with stage IV neuroblastoma: A phase II study. Prog Clin Biol Res. 1994; 385:319–328. [PubMed: 7972227]
74. Kushner BH, Kramer K, Cheung NK. Phase II trial of the anti-G(D2) monoclonal antibody 3F8 and granulocyte-macrophage colony stimulating factor for neuroblastoma. J Clin Oncol. 2001; 19:4189–4194. [PubMed: 11709561]
75. Handgretinger R, Baader P, Dopfer R, et al. A phase I study of neuroblastoma with the anti-ganglioside GD2 antibody 14.G2a. Cancer Immunol Immunother. 1992; 35:199–204. [PubMed: 1638557]
76. Murray JL, Cunningham JE, Brewer H, et al. Phase I trial of murine monoclonal antibody 14G2a administered by prolonged intravenous infusion in patients with neuroectodermal tumors. J Clin Oncol. 1994; 12:184–193. [PubMed: 8270976]
77. Frost JD, Hank JA, Reaman GH, et al. A phase I/IB trial of murine monoclonal anti-GD2 antibody 14.G2a plus interleukin-2 in children with refractory neuroblastoma: A report from the Children's Cancer Group. Cancer. 1997; 80:317–333. [PubMed: 9217046]
78. Handgretinger R, Anderson K, Lang P, et al. A phase I study of human/mouse chimeric antiganglioside GD2 antibody ch14.18 in patients with neuroblastoma. Eur J Cancer. 1995; 31A: 261–267. [PubMed: 7718335]
79. 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 refractor neuroblastoma and osteo-sarcoma. J Clin Oncol. 1998; 16:2169–2180. [PubMed: 9626218]
80. 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 Translplantation: A Children's Cancer Group Study. J Clin Oncol. 2000; 18:4077–4085. [PubMed: 11118469]
81. 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. 2009; 27:85–91. [PubMed: 19047298]
82. Osenga KL, Hank JA, Albertini MR, et al. A phase I clinical trial of the hu14.18-IL2 (EMD 273063) as a treatment for children with refractory or recurrent neuroblastoma and melanoma: A study of the Children's Oncology Group. Clin Cancer Res. 2006; 12:1750–1759. [PubMed: 16551859]
83. 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 Phase II Study. J Clin Oncol. 2010; 28:4969–4975. [PubMed: 20921469]

TABLE I

The International Neuroblastoma Risk Group Consensus Pre-Treatment Classification Schema

INRG stage	Patient age (months)	Tumor histology	Tumor differentiation	*MYCN* gene amplification	11q Aberration	DNA ploidy	Pre-treatment risk group
L1/L2	Any	GN maturing, GNB intermixed	Any	Any	Any	Any	Very Low
L1	Any	Any (except GN maturing, GNB intermixed)	Any	No	Any	Any	Very Low
MS	<18	Any	Any	No	No	Any	Very Low
L2	<18	Any (except GN maturing, GNB intermixed)	Any	No	No	Any	Low
L2	≥18	GNB nodular, neuroblastoma	Differentiating	No	No	Any	Low
M	<18	Any	Any	No	Any	Hyperdiploid	Low
L2	<18	Any (except GN maturing, GNB intermixed)	Any	No	Yes	Any	Intermediate
L2	≥18	GNB nodular, neuroblastoma	Differentiating	No	Yes	Any	Intermediate
L2	≥18	GNB nodular, neuroblastoma	Poorly Differentiated or Undifferentiated	No	Any	Any	Intermediate
M	<18	Any	Any	No	Any	Diploid	Intermediate
L1	Any	Any (except GN maturing, GNB intermixed)	Any	Yes	Any	Any	High
L2	Any	Any	Any	Yes	Any	Any	High
M	<18	Any	Any	Yes	Any	Any	High
M	≥18	Any	Any	Any	Any	Any	High
MS	<18	Any	Any	Yes	Any	Any	High
MS	<18	Any	Any	Any	Yes	Any	High

Adapted from [6]. Abbreviations: GN, ganglioneuroma; GNB, ganglioneuroblastoma.

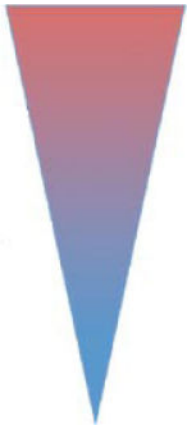
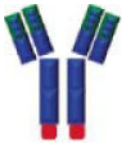
TABLE II

Gene Sets Identified by Gene Expression Profiling of Neuroblastoma Patient Samples

Author	Population	Gene set identified	Common genes	Additional findings	Comments
Wei et al. [15]	56 tumor samples	19 genes	*MYCN* upregulated, *CNR1* downregulated in poor outcome group	*DLK1* (Notch ligand) upregulated in poor outcome group	Small patient cohort
Ohira et al. [14]	136 tumor samples	41 genes	*MYCN, AHCY, TKT* upregulated in unfavorable cohort; *DDC, CLSTN1* upregulated in favorable cohort	*TUBA1, TUBA3* (tubulin members) upregulated in favorable group	Not compared with current risk stratification
Schramm et al. [16]	68 patients	39 genes	*TKT* increased in cases of relapse; *AKRIC1, NTRK1, NRCAM, CLSTN1, CHD5, DDC, MART* increased in low stage tumors	*PSMC1, PSMB5, NEDD8* (proteasomal members) increased in relapsed cases	Gene set not validated with independent patient cohort
Oberthuer et al. [17]	251 patients	144 genes	*CNR1, MAPT1, NTRK1, CAMTA1, AKRIC1, NRCAM, PTPRH, PTN* increased in favorable cases; *AHCY* increased in unfavorable cases	*CCNB2, CDC2, CDKN3* (cell cycle) increased in unfavorable cases	Limited to German patients
Asgharzadeh et al. [13]	102 patients	55 genes	*CAMTA1, CNR1, NTRK1* upregulated in low risk cases without disease progression	*USP8* (deubiquitinating enzyme) increased in low risk cases	Limited to *MYCN* nonamplified tumors
Schramm et al. [18]	63 patients	24 genes	Increased *NTRK1, PTPRF* in cases with prolonged survival	Proteasomal gene expression (*NEDD8, OSMD10*) associated with relapse	Analysis with both microarray and RT-PCR
Vermeulen et al. [11]	579 patients	59 genes	*PTN, NRCAM, DDC, NTRK1, MAPI, CHD5, AKRIC1, CAMTA1, CLSTN1, PTPRF, PTPRH* increased in low-risk cases; *MYCN, AHCY* increased in high-risk cases	*ODC1* increased in high-risk cases	Re-analysis of previously identified prognostic genes; large patient cohort from multiple international collaborative groups
Kamei et al. [19]	32 patients	283 genes		*DHRS3, NROB1, CYP26A1* overexpressed in favorable tumors	Correlation by RT-PCR for 3 genes in 121 samples
de Preter et al. [20]	730 patients	42 genes	*MYCN, AHCY* associated with poor outcomes; *AKRIC1, CLSTN1, DDC, MAPT, NRCAM, NTRK1, PTN* associated with favorable outcomes		Re-analysis of previously identified prognostic gene sets

TABLE III

Antibodies in Use for Neuroblastoma

	Antibody type	Antibody structure	Immunogenicity	GD2 antibodies used in clinical trials	References (or clinicaltrials.gov Reference Number)
	Murine 100% mouse	Mouse derived constant and variable regions		3F8 14.G2a	[29, 72–74] [75–77]
	Chimeric ~1/3 mouse and 2/3 human	Human constant and murine variable regions		ch14.18	[33, 78–81]
	Humanized ~10% mouse and 90% human	Almost all human structure, but retains murine protein sequences needed for antibody binding		hu3F8 hu14.18K3222A	NCT01419834 NCT00743496
	Chimeric immunocytokine	Chimeric antibody with a covalently linked cytokine at the C terminus			
	Humanized immunocytokine	Humanized antibody with a covalently linked cytokine at the C terminus		hu14. 18-IL2	[82, 83]