

Pediatric Solid Tumors: Dose Intensity and Targeted Therapy

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Introduction

Solid tumors make up 56% of all childhood cancers diagnosed annually in the United States. The main categories are astrocytoma (9.6%), neuroblastoma and ganglioneuroblastoma (7.9%), Wilm's tumor (6.4%), medulloblastoma (4.2%), rhabdomyosarcoma (3.6%), germ cell tumor (3.2%), osteosarcoma (2.6%), and Ewing's sarcoma (2%).⁽¹⁾ Treatment methods generally include combination chemotherapy and combined modality strategies. For certain cancers such as Wilm's tumor, cure is achievable in the large majority of patients. Unfortunately, most metastatic solid tumors and high-grade malignant brain tumors have remained resistant to treatment, despite the tremendous physical, psychosocial and economical toll to the patient and the patient's family.

In the last decade, consensus on staging systems has been reached for most pediatric solid tumors. This was accompanied by improved staging methods, better definitions of risk groups, and cooperation among treatment centers as in POG (Pediatric Oncology Group) and CCG (Children's Cancer Group) in the U.S.⁽²⁾ Increasing attention is being paid to the late effects of treatment.⁽³⁾ More recently, the discovery of unique chromosomal aberrations (Table 1), cellular oncogenes (e.g., MYCN in neuroblastoma),⁽⁴⁾ tumor suppressor genes (Table 2), as well as autocrine growth factors and their receptors (e.g., nerve growth factor [NGF] and brain derived nerve growth factor [BDNF], their receptors TrkA and TrkB, respectively, in neuroblastoma)⁽⁴⁾ has furthered our understanding of the complex nature of malignant transformation and may provide molecular/biochemical targets for tumor-selective therapy.⁽⁵⁾ Combining the specificity of immunologic vehicles (antibodies or cells) and the potential of gene manipulation, more effective and less toxic cancer treatment modalities may be possible.⁽⁶⁻⁸⁾

Table 1. Chromosomal aberrations⁽⁵⁾

Tumor	Chromosomal Aberrations	Chimeric Gene	Estimated Frequency
Rhabdomyosarcoma	t(2;13)(q35;q14)	PAX3-FKHR	95%
	t(1;13)(p36;q14)	PAX7-FKHR	5%
Ewing's/PNET	t(11;22)(q24;q12)	EWS-FL1	95%
	t(21;22)(q22;q12)	EWS-ERG	2%
	t(7;22)(p22;q22)	EWS-ETV1	2%
Desmoplastic small	t(11;22)(p13;q12)	EWS-WT1	unknown

round cell sarcoma

Table 2. Tumor suppressor genes⁽⁵⁾

Tumor	Chromosomal Site	Suppressor	Protein Gene	
Retinoblastoma	13q14		RB	110kD regulator of G1→S cell cycle transition
Glioma, sarcoma	17p13		p53	53 kD transcription factor, regulates G1→S checkpoint
PNET, glioma neuroblastoma	17q11		NF1	327 kD stimulator of ras-specific GTPase
Astrocytoma	22q12		NF2	66 kD (membrane-cytoskeleton interface)
Astrocytoma, glioma, osteosarcoma	9p21		p16INK4A	16 kD inhibitor of cyclinD-CDK4 or CDK6 complex
Wilm's tumor	11p13		WT1	45 kD transcription factor (zinc-finger motif)
Neuroblastoma	1p36		CDC2L1	58 kD CDK-related kinase (PITSLRE)

Neuroblastoma

Neuroblastoma arises from neural crest cells that are progenitors of the adrenal medulla and the sympathetic nervous system. It is the most common extracranial solid tumor of childhood and the most common neoplasm diagnosed in the first year of life.⁽¹⁾ Ninety percent of cases are diagnosed in children 5 years old or less, with a median age of 2.5 years. The diagnosis is established by characteristic histopathologic findings or by tumor cell clumps in bone marrow plus elevations in urinary catecholamines such as vanillylmandelic acid; occasionally, more sophisticated techniques are required to rule out other small round cell tumors such as Ewing's sarcoma (ES), primitive neuroectodermal tumor (PNET), rhabdomyosarcoma and occasionally lymphoma.⁽⁴⁾ Unlike other solid tumors, 60% of neuroblastoma is already metastatic at the time of diagnosis. Massive primary tumors and metastatic disease give neuroblastoma its dismal reputation because, despite experiencing marked tumor regressions with cytotoxic therapy, most of these patients eventually die of disease. Nevertheless, the application of

rational treatment principles (e.g., dose intensity) as well as the use of novel targeted therapy may change the natural history of this recalcitrant disease.

Dose Intensity⁽⁹⁾

Early combination chemotherapy regimens using low doses of cyclophosphamide, doxorubicin, vincristine and agents (e.g., DTIC) with less activity against neuroblastoma produced modest response rates in patients with stage 4 neuroblastoma. The median duration from diagnosis to disease progression was approximately eight months, and few patients were cured. Despite dramatic disease regressions during induction, the clinical emergence of drug resistance has been the frustrating hallmark of treating neuroblastoma. Drug resistance is a complex phenomenon largely related in part to the enormous tumor burden characteristic of stage 4 neuroblastoma. Goldie and Coldman proposed a model for understanding and hence for approaching the problem of drug resistance in cancer.⁽¹⁰⁾ This model, which appears to be applicable to neuroblastoma, posits that spontaneous mutations confer drug resistance and that the number of drug-resistant clones is a function of tumor size, the rate of mitosis and a permissive (i.e., subtherapeutic) environment. Rapid elimination of sensitive cells may reduce the development of drug-resistant mutants. The model predicts that the initiation at diagnosis of maximal dosing and dose-rate, using active and noncross-resistant chemotherapeutic agents, should yield improved clinical results. Support for early dose-intensive use of chemotherapy also comes from a retrospective analysis of 44 published neuroblastoma clinical trials.⁽¹¹⁾ This study showed that major response rates, median survival, and progression-free survival correlated strongly with the amount of four drugs (cyclophosphamide, doxorubicin, cisplatin, epipodophyllotoxins) given during the first 21 weeks following diagnosis. Vincristine and DTIC dose intensities did not contribute to improved clinical outcome. Alkylating agents, including cyclophosphamide, constitute a large class of effective agents against neuroblastoma. Features accounting for the clinical efficacy of alkylating agents include their cell-cycle nonspecificity, their activity against hypoxic as well as oxygenated tumor cells, and their tissue penetration—Ñall important attributes when treating large tumors with poor vascular perfusion and relatively dormant subpopulations of tumor cells.⁽¹²⁾ Properties of cyclophosphamide that set it apart from other alkylators in terms of potential for maximizing dose intensity include its negligible extramedullary toxicity (in contrast to ifosfamide, for example) and its relative sparing of hemopoietic stem cells (in contrast to melphalan, thiotepa, busulfan, and carmustine). Multiple courses of high-dose cyclophosphamide can therefore be administered within a short time frame, which translates into increased dose intensity. In addition, this use of cyclophosphamide does not preclude subsequent harvesting of bone marrow for future transplantation. Alkylating agents have a log-linear dose-response effect against tumor cells in vitro that is maintained through four to five logs of tumor cell kill, providing an experimental rationale for maximizing dose intensity. Enhanced chemotherapeutic efficacy may be achieved, not only through escalating dosage and increasing dose-rate, but also through modulating mechanisms of drug-resistance operative at the cellular level. These include depletion of intracellular glutathione by buthionine and sulfoximine, and modulation of P-glycoprotein and multidrug resistance protein functions.^(13,14)

Using dose-intensive induction therapies, > 90% of patients diagnosed at more than 1 year of age with metastatic stage 4 neuroblastoma are expected to achieve complete or near-complete remissions. However, the successful eradication or control of minimal residual disease remains the final therapeutic challenge. Myeloablative therapy followed by autologous marrow rescue (ABMT) has been widely used in recent years. Alkylating agents, in particular melphalan, remain the centerpiece of transplant programs for neuroblastoma. A major weakness of autografting in patients with neuroblastoma is contamination by occult tumor cells. That contaminated marrow is at least partly responsible for marrow relapse is clearly shown by gene-marking experiments.⁽¹⁵⁾ The high frequency of bone marrow invasion by neuroblastoma has prompted the routine use of ex vivo immunologic or pharmacologic purging of harvested bone marrow. Peripheral blood stem cell rescue has been widely applied in myeloablative therapies for various malignancies. However, peripheralization of clonogenic neuroblasts is common in neuroblastoma,⁽¹⁶⁾ and ex vivo purging is necessary. Although ABMT has prolonged survival, its precise contribution is obscured by concurrent improvements in induction chemotherapy. Two retrospective analyses did not show a statistical benefit between ABMT versus continued chemoradiotherapy.^(17,18) The only randomized study reported to date found that high-dose melphalan prolonged event-free survival, but probably did not increase the overall cure rate.⁽¹⁹⁾ Indeed, a similar conclusion emerges from reviews of unselected patients who were consecutively entered at diagnosis on treatment protocols consisting of moderate-dose combination chemotherapy plus myeloablative regimens for consolidation of remission.^(20,21) Although event-free survival is prolonged in comparison to historical controls, it declines steadily with time, reaching only 12%-18% in the studies that had the largest number of patients and the longest follow-up. Length of follow-up is important because relapses are not uncommon in patients who have been off all therapy and are in complete remission for more than two years.⁽²⁰⁾ In the U.S., the Children's Cancer Study Group is conducting a prospective randomized study with the aim of comparing the efficacy of myeloablative treatment versus intensified conventional chemotherapy in poor risk neuroblastoma. The cost versus benefit ratio of ABMT, especially when compared to other less toxic approaches, needs to be definitively resolved.

Monoclonal Antibody Targeted Immunotherapy⁽⁸⁾

Monoclonal antibodies (MoAb) can mediate efficient tumor cell kill by human complement and by human lymphocytes, neutrophils and activated monocyte/macrophages. Neuroblastoma has poor expression of decay-accelerating factor and is thus unable to resist complement activation and cytotoxicity. Complement may be critical for the initiation of inflammation, which can attract leukocytes and increase vascular permeability to proteins, including passively administered monoclonal antibodies. In neuroblastoma, only a limited number of target antigens that are tumor-selective have been described. Most are glycoproteins, to which only a few antibodies have been tested clinically. The glycolipid antigen ganglioside GD2 is abundantly expressed in neuroblastoma, to which several antibodies have been made. These include 14.G2a (a mouse IgG2a class switch variant from 14.18), ch14.18 (a human-mouse

chimeric form of 14.18) and 3F8 (mouse IgG3). Disialoganglioside GD2 is well suited for targeting therapy because ⁽¹⁾ it is expressed at a high density in human neuroblastoma ($5-10 \times 10^6$ molecules per cell) and is restricted to neuroectodermal tissues; ⁽²⁾ although it circulates in patients' serum, it does not interfere with the biodistribution of specific antibody (e.g., 3F8), allowing excellent tumor localization of neuroblastomas in patients; ⁽³⁾ characteristic of carbohydrate antigens, GD2 is not modulated from cell surface upon binding to antibodies; ⁽⁴⁾ it is expressed homogeneously in human neuroblastoma, with little heterogeneity within tumors and among patients.

A number of phase I/II studies of anti-GD2 monoclonal antibodies have been carried out to define the acute toxicities and efficacy of unconjugated antibodies. At dose levels of 5 mg/m² to 400mg/m² there were significant toxicities, including severe pain, hypertension, hypotension, hyponatremia, sensory and autonomic neuropathy. Anti-tumor activity has been observed in patients with chemorefractory neuroblastomas and melanomas, best demonstrated for marrow disease. The formation of human anti-mouse antibodies (HAMA) and the inefficiency of most murine IgG subclasses in binding to human Fc receptors have early on stimulated a large effort in genetically engineering chimeric and humanized antibodies. At Memorial Sloan-Kettering Cancer Center, the role of anti-GD2 antibody as an adjuvant was analyzed among patients with high-risk neuroblastoma (stage 4, diagnosed after 20 months of age, with bone and marrow disease, who re-achieved near-complete clinical remission following progression off therapy). More than 30% of these children have remained progression-free without other systemic therapy from 2 to 8 years following MoAb treatment. Although the anti-tumor effect appears to be associated with complement-mediated cytotoxicity (CMC) and antibody-dependent cell-mediated-cytotoxicity (ADCC), additional mechanisms are likely, given the length of time required for response (weeks after the initiation of treatment) and continued response long after treatment has ended. One hypothesis is the induction of an anti-idiotypic network. Patients who developed anti-idiotypic⁽²²⁾ or anti-anti-idiotypic antibodies⁽²³⁾ demonstrated more responses or longer progression-free survivals. These observations suggest that MoAb-mediated anti-tumor effect may proceed in at least two phases. While the initial rapid phase utilizes CMC and ADCC, the second delayed phase may involve an anti-anti-idiotypic response.

Radioimmunotherapy⁽⁹⁾

The radiosensitivity of neuroblastoma, plus the unsatisfactory results with chemotherapy alone, has stimulated efforts to develop systemic radiotherapeutic approaches to stage 4 neuroblastoma. These include both external beam total body irradiation (TBI) and targeted radiotherapy using radiolabeled tumor-seeking agents such as Meta-iodo-benzyl-guanidine (MIBG) or MoAbs. Early studies of hemi-body or total body external beam irradiation, using nonmyeloablative doses, did not improve results obtained with chemotherapy alone. A decade of experience in ABMT shows approximately comparable event-free survival for patients undergoing myeloablative consolidation of first remission, whether they did or did not receive TBI (generally 1000 cGy). This result can be explained by inadequate radiation dosage to tumor cells, as well

as by increased mortality from radiation-related toxicities such as veno-occlusive disease of the liver.

Targeted radioimmunotherapy⁽²⁴⁾ exploits the specificity of antibodies to deliver radioisotope (e.g., ¹³¹I) directly to tumor cells. ¹³¹I-labeled anti-GD2 MoAb localized effectively to primary neuroblastomas of the mediastinum and abdomen, as well as metastatic disease in the lymph nodes, bone marrow and bone. The specificity has been validated by tumor and marrow biopsies, as well as by CT/MRI and bone scans. A comparison with ¹³¹I-MIBG suggests that ¹³¹I-3F8 was more sensitive in detecting metastatic sites of disease. The radiological toxicities of ¹³¹I-3F8 were defined in a phase I dose escalation study. Twenty-four patients with refractory neuroblastoma (23 stage 4, 1 stage 3) were treated with ¹³¹I-3F8 at seven dose levels (6-28 mCi/kg). Twenty-two patients were rescued with cryopreserved autologous bone marrow; one patient received GM-CSF; one died of progressive disease before marrow reinfusion. Acute toxicities of ¹³¹I-3F8 treatment included pain during the infusion, fever and mild diarrhea. All patients developed grade 4 myelosuppression. Thyroid uptake despite oral SSKI led to hypothyroidism in four patients. Subsequent patients were treated with synthroid or Cytomel to suppress thyroid functions. Six patients survived > 20 months from the time of ¹³¹I-3F8 treatment; none encountered late extramedullary toxicities. Responses were seen in both soft tissue masses and bone marrow. Dose estimates based on conjugate planar images and plasma activity over time were favorable; the average dose to body organs and marrow were 1.9-2.5 and to tumor 13.7 rad/mCi, respectively.

Conclusion

The use of dose-intensive induction, radioimmunotherapy and adjuvant antibody therapy has been incorporated into the current protocol at Memorial Sloan-Kettering Cancer Center. Preliminary results suggest that the response rate, survival and progression-free survival of children with metastatic neuroblastoma have improved. There is optimism that the life expectancy of children diagnosed with high-risk neuroblastoma can be extended. The continuation of research will lead to a better understanding of the idiotype network and the development of more efficient vehicles for radioimmunotherapy. Genetic engineering approaches have great potential for building better carriers to deliver toxins, radioisotopes, genes and drugs. The era of “smart bombs” and “gene guns” has arrived.⁽²⁵⁾ These tools will offer novel strategies that attack tumors at their unique molecular targets, while sparing innocent bystander cells of the body.^(7,26-30)

References

1. Miller RW, Young JL, Novakovic B: Childhood cancer. *Cancer* 75:395, 1994
2. Pediatric Oncology Group: Progress against childhood cancer: The Pediatric Oncology Group experience. *Pediatrics* 89:597, 1992
3. Bhatia S, Robison LL, Oberlin O, Greenberg M, Bunin G, Fossati-Bellani F, Meadows AT: Breast cancer and other second neoplasms after childhood Hodgkin's disease. *N Engl J Med* 334:745, 1996

4. Brodeur GM, Castleberry RP: Neuroblastoma, in Pizzo PA, Poplack DG (eds): Principles and Practice of Pediatric Oncology (ed 2). Philadelphia, J.B. Lippincott Company, 1993, p 739
5. Dai Y, Schwarz EM, Gu D, Zhang WW, Sarvetnick N, Verma IM: Cellular and humoral immune responses to adenoviral vectors containing factor IX gene: Tolerization of factor IX and vector antigens allows for long-term expression. Proc Am Soc Clin Oncol 92:1401, 1995
6. Wagner T, Zink M, Shwieder G: Influence of mesna and cysteine on the systemic toxicity and therapeutic efficacy of activated cyclophosphamide. J Cancer Res Clin Oncol 113:160, 1987
7. Hwu P: The gene therapy of cancer. PPO Updates 9:1, 1995
8. Cheung NKV: Biological and molecular approaches to diagnosis and treatment. Section I. Principles of immunotherapy, in Pizzo PA, Poplack DG (eds): Principles and Practice of Pediatric Oncology (ed 3). Philadelphia, J.B. Lippincott Company, 1996 (in press)
9. Kushner BH, Cheung NKV: Neuroblastoma: an overview. Hem/Onc Annals 1:189, 1993
10. Goldie JH, Coldman AJ: The somatic mutation theory of drug resistance: The "Goldie-Coldman" hypothesis revisited, in Devita HR (ed): Cancer. Principles and Practice of Oncology (ed 3, vol 3). Philadelphia, PA, JP Lippincott, 1989, p 1
11. Cheung NK, Heller G: Chemotherapy dose intensity correlates strongly with response, median survival and median progression-free survival in metastatic neuroblastoma. J Clin Oncol 9:1050, 1991
12. Frei E III, Teicher BA, Holden SA, Cathcart KNS, Wang Y: Preclinical studies and clinical correlation of the effect of alkylating dose. Cancer Res 48:6417, 1988
13. Goldstein LJ, Fojo AT, Ueda K, Crist W, Green A, Brodeur G, Pastan I, Gottesman MM: Expression of the multidrug resistance, MDR1, gene in neuroblastomas. J Clin Oncol 8(1):128, 1990
14. Norris MD, Bordow SB, Marshall GM, Haber PS, Cohn SL, Haber M: Expression of the gene for multidrug-resistance-associated protein and outcome in patients with neuroblastoma. N Engl J Med 334:231, 1996
15. Brenner MK, Rill DR, Holladay MS, Heslop HE, Moen RC, Buschle M, Krance RA, Santana VM, Anderson WF, Ihle JN: Gene marking to determine whether autologous marrow infusion restores long-term haemopoiesis in cancer patients. Lancet 342:1134, 1993
16. Moss TJ, Cairo M, Santana VM, Weinthal J, Hurvitz C, Bostrom B: Clonogenicity of circulating neuroblastoma cells: Implications regarding peripheral blood stem cell transplantation. Blood 83:3085, 1994
17. Shuster JJ, Cantor AB, McWilliams N, Graham-Pole J, Castleberry RP, Marcus R, Pick T, Smith EI, Hayes FA: The prognostic significance of autologous bone marrow transplant in advanced neuroblastoma. J Clin Oncol 9:1045, 1991
18. Stram DO, Matthay KK, O'Leary M, Reynolds CP, Seeger RC: Myeloablative chemoradiotherapy versus continued chemotherapy for high risk neuroblastoma, in Evans AE, Biedler JL, Brodeur GM, D'Angio GJ, Nakagawara A (eds): Advances in Neuroblastoma Research 4 (vol 385). New York, Wiley-Liss, 1994, p 287

19. Pinkerton CR, Hartmann O, Dini G, Philip T: ENSG 1 - Randomized study of high-dose melphalan in neuroblastoma, in Dicke KA, Spitzer G, Jagannath S (eds): Autologous Bone Marrow Transplantation - Proceedings of the Third International Symposium. Houston, The University of Texas M.D. Anderson Hospital and Tumor Institute, 1989, p 401
20. Phillip T, Zucker JM, Bernard JL, Lutz BP, Bordigoni P, Plouvier E, Robert A, Roche H, Souillet G, Bouffet E, Michon J, Lopez M, Vilcoq JM, Gentet JC, Philip I, Ladenstein R, Favrot M, Chauvin F: Improved survival at 2 and 5 years in the LMCE1 unselected group of 72 children with stage IV neuroblastoma older than 1 year of age at diagnosis: Is cure possible in a small subgroup? *J Clin Oncol* 9(6):1037, 1991
21. Hartmann O, Benhamou E, Beaujean F, Kalifa C, Lejars O, Patte C, Behard C, Flamant F, Thyss A, Deville A, Vannier JP, Pautard-Muchemble B, Lemerle J: Repeated high-dose chemotherapy followed by purged autologous bone marrow transplantations as consolidation therapy in metastatic neuroblastoma. *J Clin Oncol* 5:1205, 1987
22. Murray JL, Cunningham JE, Brewer H, Mujoo K, Zukiwski AA, Podoloff DA, Kasi LP, Bhadkamkar V, Fritsche HA, Benjamin RS, Legha SS, Ater JL, Jaffe N, Itoh K, Ross MI, Bucana CD, Thompson L, Cheung L, Rosenblum MG: Phase I trial of murine monoclonal antibody 14G2a administered by prolonged intravenous infusion in patients with neuroectodermal tumors. *J Clin Oncol* 12:184, 1994
23. Cheung NK, Cheung IY, Canete A, Yeh SJ, Kushner BH, Bonilla MA, Heller G, Larson SM: Antibody response to murine anti-GD2 monoclonal antibodies: Correlation with patient survival. *Cancer Res* 54:2228, 1994
24. Larson SM, Sgouros G, Cheung NK: Antibodies in cancer therapy: Radioisotope conjugates, in DeVita VT, Hellman S, Rosenberg SA (eds): *Biologic Therapy of Cancer* (ed 2). Philadelphia, J.B. Lippincott Co., 1995, p 534
25. Uckun FM, Evans WE, Forsyth CJ, Waddick KG, Ahlgren LT, Chelstrom LM, Burkhardt A, Bolen J, Myers DE: Biotherapy of B-cell precursor leukemia by targeting Genistein to CD19-associated tyrosine kinases. *Science* 267:886, 1995
26. George AJT, Spooner RA, Epenetos AA: Applications of monoclonal antibodies in clinical oncology. *Immunol Today* 15(12):559, 1994
27. Guo HF, Rivlin K, Dubel S, Cheung NKV: Recombinant anti-ganglioside GD2 scFv-streptavidin fusion protein for tumor pretargeting. *Proc Am Assoc Cancer Res* 37:469, 1996 (abstract)
28. Brenner MK. The application of gene transfer to pediatric malignant disease. In: *Principles and Practice of Pediatric Oncology* (ed 3). Pizzo PA, Poplack DG (eds). Philadelphia, JB Lippincott Co, 1996 (in press)
29. Hwu P, Rosenberg SA: Gene therapy using lymphocyte modification, in DeVita Jr. VT, Hellman S, Rosenberg SA (eds): *Biologic Therapy of Cancer* (ed 2). Philadelphia, J.B. Lippincott Company, 1995, p 727
30. Chen SH, Shine HD, Goodman JC, Grossman RG, Woo SLC: Gene therapy for brain tumors: Regression of experimental gliomas by adenovirus-mediated gene transfer in vivo. *Proc Natl Acad Sci (USA)* 91:3054, 1994