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Hot Topic

Neuroblastoma: Therapeutic strategies for a clinical enigma

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SUMMARY

Neuroblastoma, the most common extracranial pediatric solid tumor remains a clinical enigma with outcomes ranging from cure in >90% of patients with locoregional tumors with little to no cytotoxic therapy, to <30% for those >18 months of age at diagnosis with metastatic disease despite aggressive multimodality therapy. Age, stage and amplification of the *MYCN* oncogene are the most validated prognostic markers. Recent research has shed light on the biology of neuroblastoma allowing more accurate stratification of patients which has permitted reducing or withholding cytotoxic therapy without affecting outcome for low-risk patients. However, for children with high-risk disease, the development of newer therapeutic strategies is necessary. Current surgery and radiotherapy techniques in conjunction with induction chemotherapy have greatly reduced the risk of local relapse. However, refractory or recurrent osteomedullary disease occurs in most patients with high-risk neuroblastoma. Toxicity limits for high-dose chemotherapy appear to have been reached without further clinical benefit. Neuroblastoma is the first pediatric cancer for which monoclonal-antibody-based immunotherapy has been shown to be effective, particularly for metastatic osteomedullary disease. Radioimmunotherapy appears to be a critical component of a recent, successful regimen for treating patients who relapse in the central nervous system, a possible sanctuary site. Efforts are under way to refine and enhance antibody-based immunotherapy and to determine its optimal use. The identification of newer tumor targets and the harnessing of cell-mediated immunotherapy may generate novel therapeutic approaches. It is likely that a combination of therapeutic modalities will be required to improve survival and cure rates.

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Introduction

Clinical and laboratory research over the last three to four decades has shed considerable light on the biology of neuroblastoma (NB), the most common extracranial tumor of childhood. However, it remains one of the most enigmatic solid tumors for pediatric oncologists. On one hand, tumors may regress completely or differentiate into benign ganglioneuroblastoma without treatment, while on the other, metastatic NB in children >18 months of age at diagnosis is lethal for most patients despite aggressive multimodality therapy. Early tumor detection by screening did not reduce tumor-related mortality[1,2] and low stage tumors with favorable biological markers often do not metastasize even when incompletely resected. Conversely, high-risk NB is sensitive to dose-intensive chemotherapy: a majority of patients achieve remission after induction chemotherapy, surgery and radiotherapy; but most relapse even with consolidation therapy. Rates of tumor progression and response are often age dependent: adolescents and adults usually have indolent, chemoresistant tumors compared to NB in younger children.[3,4]

This clinical heterogeneity has fascinated investigators and has led to research into numerous potential markers for stratification and prognostication and into the detection of tumor antigens and pathways that can be targeted by newer therapeutic agents. Amplification of the *MYCN* oncogene, first described in NB patients[5] in 1983 was one of the first genes shown to be of prognostic value in pediatric oncology and *MYCN*-amplification maintains its relevance as a marker of high-risk disease for patients with locoregional NB. The definition of *MYCN*-amplification has since been refined as $\geqslant$10 gene copies per diploid genome; tumors with 3–10 copies do not behave like *MYCN*-amplified tumors.[6] To date, age and stage remain the most validated clinical prognostic markers for patients with NB. The international NB staging system (INSS) established in 1989 is currently used to stage patients with NB[7] (Table 1). However, a new presurgical International Neuroblastoma Risk Group (INRG) Staging System was recently proposed incorporating presurgical image defined risk factors.[8] An INRG task force has further proposed classifying NB patients into 16 risk groups by establishing a pre-treatment INRG classification system (INRGCS) that will utilize biological markers such as *MYCN*-amplification, ploidy, histologic assessment and chromosome 11q status in addition to age and stage[9] (Table 2). The significance of the

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Table 1
International neuroblastoma staging system.[166]

Stage 1	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached and removed with the primary tumor may be positive)
Stage 2A	Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically
Stage 2B	Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically
Stage 3	Unresectable unilateral tumor infiltrating across the midline[a], with or without regional lymph node involvement; or localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement
Stage 4	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined for stage 4S)
Stage 4S	Localized primary tumor (as defined for stage 1, 2A, or 2B), with dissemination limited to skin, liver and/or bone marrow[b] (limited to infants <1 year of age)

Multifocal primary tumors (e.g., bilateral adrenal primary tumors) should be staged according to the greatest extent of disease, as defined previously, followed by subscript "M".

[a] The midline is defined as the vertebral column. Tumors originating on one side and "crossing the midline" must infiltrate to or beyond the opposite side of the vertebral column.

[b] Marrow involvement in stage 4S should be minimal, that is, less than 10% of total nucleated cells identified as malignant on bone marrow biopsy or on marrow aspirate. More extensive marrow involvement would be considered to be stage 4. The MIBG scan (if done) should be negative in the marrow.

Table 2
International neuroblastoma risk group (INRG) consensus pre-treatment classification system.[9]

INRG stage	Age (months)	Histologic category	Grade of tumor differentiation	*MCYN*	11q aberration	Ploidy	Pre-treatment risk group
L1/L2		GN maturing GNB intermixed					A Very low
L1		Any except GN maturing GNB intermixed		NA			B Very low
				Amp			K High
L2	<18	Any except GN maturing GNB intermixed		NA	No		D Low
					Yes		G Intermediate
	⩾18	GNB nodular; neuroblastoma	Differentiating	NA	No		E Low
					Yes		H Intermediate
			Poorly differentiating or undifferentiated	NA			
				Amp			N High
M	<18			NA		Hyperdiploid	F Low
	<12			NA		Diploid	I Intermediate
	12 to <18			NA		Diploid	L Intermediate
	<18			Amp			O High
	⩾18						P High
MS	<18			NA	No		C Very low
					Yes		Q High
				Amp			R High

Amp: amplified; GN: ganglioneuroma; GNB: ganglioneuroblastoma; NA: non-amplified; INRG stages L1, L2, M and MS are defined by INRG staging system.[8]

INRGCS will require validation in prospective clinical studies. The current Memorial Sloan-Kettering Cancer Center (MSKCC) strategies and outcomes for patients with NB are summarized in Fig. 1. We discuss below current approaches to treatment of locoregional and metastatic NB.

Locoregional NB

About 50% of patients do not have evidence of metastatic disease at diagnosis, i.e., have INSS stage 1, 2 or 3 disease.[10] Most of these patients are considered to have low-risk NB. An exception is the group of patients with tumors that are *MYCN*-amplified.

Non-MYCN-amplified locoregional NB

Patients with non-*MYCN*-amplified INSS stage 1 can be cured with surgical resection alone.[11] However patients with non-*MYCN*-amplified stage 2 and 3 NB have been treated with various regimens ranging from (1) observation alone in infants[12] to (2) surgery without any cytotoxic therapy[13,14] to (3) intermediate-dose chemotherapy[15] to (4) high-dose chemotherapy plus myeloablative chemotherapy with autologous hematopoietic stem-cell rescue (ASCT);[16–18]. The rationale for the first strategy is based on the lack of progression of non-stage 4 tumors discovered by screening programs, the limited proliferative and metastatic potential of residual post-operative disease, the severity of the long-term side effects of cytotoxic therapy[19], the relative lack of chemoresponsiveness of some localized NB, and the curability of recurrent low-risk localized NB.[20] Utilizing such an approach at MSKCC, we recently reported an 84.6 ± 14% 10-year overall survival (OS) for patients with non-*MYCN*-amplified stage 3 NB treated without any cytotoxic chemotherapy regardless of histological classification, DNA index or serum ferritin.[21] Outcome for stage 1 and 2 tumors has been similarly favorable. Equally promising results have been reported by other small single institution studies treating INSS stage 2 (or Evans stage II) patients without cytotoxic therapy.[22,23] In contrast to the MSKCC approach, the children's oncology group (COG) currently considers all stage 3 patients <574 days old at diagnosis and >574 days old with favorable histology (depending on the degree of neuroblast differentiation, Schwannian stroma content and mitosis-karyorrhexis index) to have intermediate risk disease.[24] Such patients have been reported to have a favorable outcome with low dose chemotherapy without radiotherapy.[25] Stage 3 patients >574 days of age with unfavorable histology are considered to have high-risk disease. Data from children's cancer group (CCG) protocol 3891 indicate 5-year OS of 70 ± 9% for the latter group when treated with chemotherapy with or without ASCT.[26] The MSKCC approach that

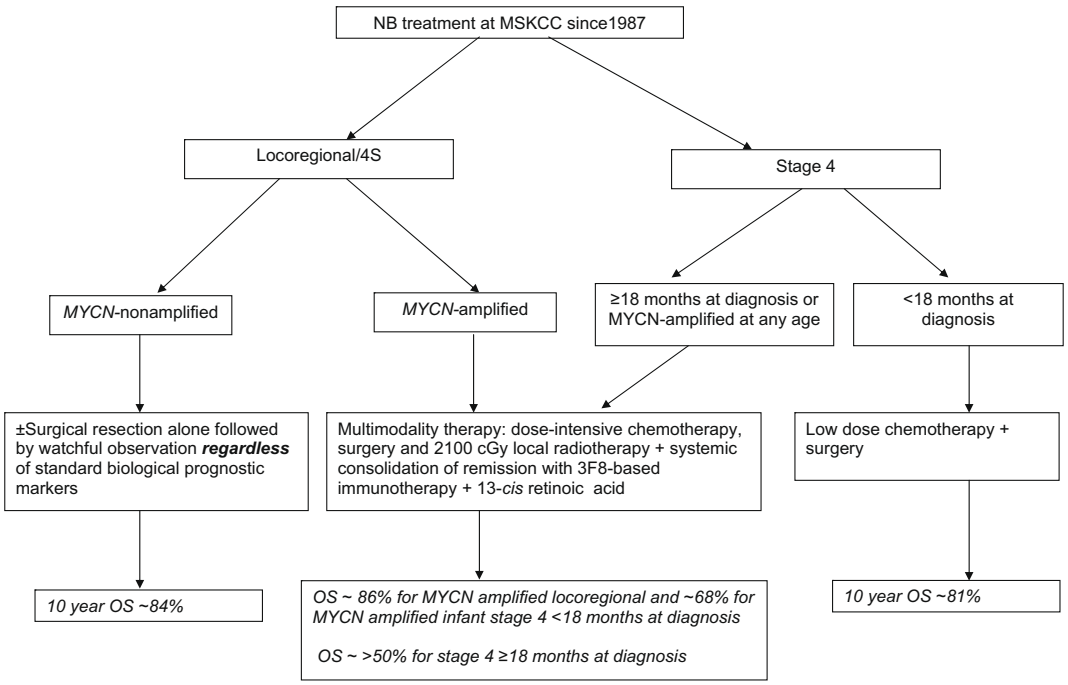

Fig. 1. Memorial Sloan-Kettering Cancer Center approaches and outcomes in patients with locoregional and metastatic neuroblastoma.

withholds cytotoxic therapy chemotherapy or discontinues all such therapy after surgery yields similar favorable results (100% 10-year OS) suggesting that the role of dose-intensive chemotherapy for this group of patients is not firmly established.[21] A retrospective analysis of the large (n = 8800) INRG database did not reveal histology or DNA ploidy to be independent prognostic markers for patients with INSS stage 3 patients. The only prognostic markers that were independently prognostic in multivariate analysis were serum ferritin >92 pg/ml at diagnosis for children >18 months and chromosome 11q aberrations for children >18 months.[27] Other large retrospective studies have reported 11q and 17q abnormalities to be poor prognostic markers for NB.[28,29] In future proposed multicenter studies, the significance of these aberrations will be determined in a prospective manner.

Two uncommon clinical syndromes that are usually associated with locoregional NB (though may occasionally occur with high-risk disease) bear mentioning: (a) cord compression caused by epidural NB may lead to devastating neurological sequelae. The optimum treatment is not established[30]: early neurosurgery to relieve cord compression may prevent onset or progression of paraparesis, while increasing the risk of subsequent scoliosis.[31] Conversely, chemotherapy or radiotherapy may not act quickly enough to prevent neurological compromise but may be associated with a lower rate of spinal deformities. Treatment decisions may require consideration of individual clinical scenarios. (b) The rare paraneoplastic syndrome of opsoclonus–myoclonus syndrome, while associated with low stage NB can be associated with significant motor, behavioral and sleep disturbances. Recent reports on the possible therapeutic efficacy of rituximab[32,33] are encouraging. It has the potential to be used as an adjunct to established therapy with ACTH and intravenous immunoglobulin and may permit reduction in steroid use for this chronic condition.

MYCN-amplified locoregional NB

Locoregional *MYCN*-amplified NB has a worse prognosis than its non-*MYCN*-amplified counterpart. Internationally, patients treated on various regimens from 1991 to 2002 with *MYCN*-amplified stage 1 and 2 NB had a poorer prognosis than those without *MYCN*-amplification, though patients with hyperdiploidy appeared to fare better than those with diploid tumors.[34] Patients with stage 3 *MYCN*-amplified NB treated on CCG-3891 (n = 24) had a poorer prognosis (5-year event free survival [EFS] 25 ± 9%) despite receiving ASCT and 13-cis-retinoic acid.[26] However, the MSKCC experience for these patients appears to be favorable with a 10-year EFS and OS of 90.9 ± 8.7% using higher dose induction chemotherapy and anti-disialoganglioside (GD2) immunotherapy with the monoclonal-antibody (MoAb) 3F8 (n = 11) with or without ASCT.[21] Results for this group of patients treated on COG protocol A3973 that uses similar induction chemotherapy followed by ASCT are awaited.

Stage 4S NB

Infants with localized primary tumors with NB dissemination limited to skin, liver and mild (<10%) bone marrow without bone involvement often have disease that resolves spontaneously without therapy and have a favorable outcome, though extensive liver involvement may initially lead to cardiorespiratory compromise.[35,36] A similar "wait and watch" strategy may also be successful for some infants with localized tumors that do not meet the stage 4S definition.[12] Tumors are often hyperdiploid,[37] but at present, due to the rarity of the entity, it cannot be completely characterized biologically, though attempts have been made to identify genetic signatures that may be predictive.[38]

Stage 4 NB <18 months of age

For biological reasons that are as yet unclear, children who are <18 months old at diagnosis and have tumors that are not MYCN-amplified have a far superior prognosis when compared to older patients. The favorable prognosis for children <12 months of age with non-*MYCN*-amplified tumors has been well-established. Recent studies have extended the age for reported favorable outcomes to 18 months of age[39,40] (Fig. 2) and the current recommendations are to treat this group of patients with intermediate-dose chemotherapy without ASCT.

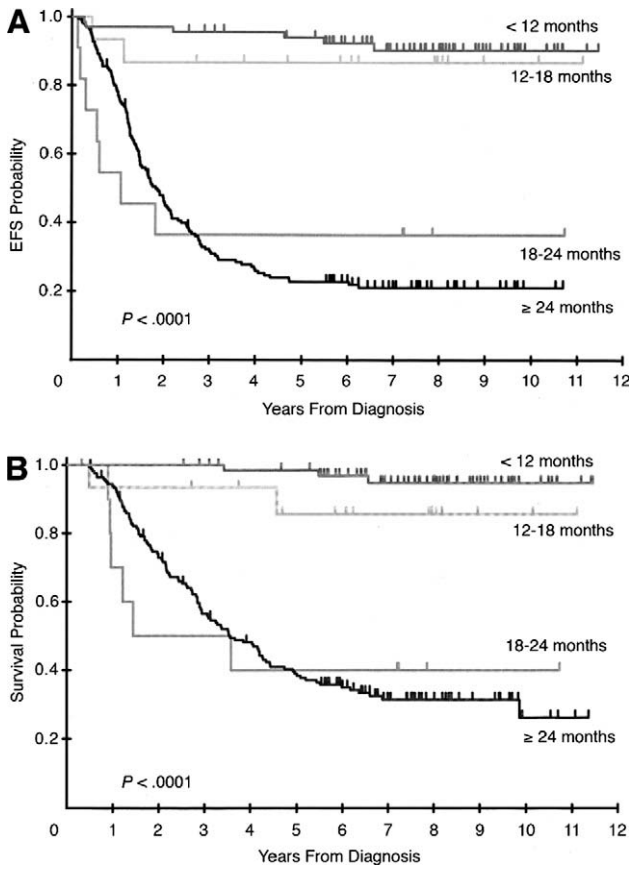

Fig. 2. Event-free survival (EFS) (A) and overall survival (OS) (B) for children with MYCN-non-amplified stage 4 neuroblastoma treated on children's cancer group (CCG) protocols CCG-3881 (for patients <12 months at diagnosis) and CCG-3891 (patients >12 months at diagnosis). (Reprinted with permission from Schmidt ML, Lal A, Seeger RC, et al. Favorable prognosis for patients 12–18 months of age with stage 4 non-amplified MYCN neuroblastoma: a children's cancer group study. J Clin Oncol 2005;23:6474–80.)

High-risk metastatic NB

Patients >18 months of age with stage 4 NB and those <18 months of age with *MYCN*-amplified stage 4 disease constitute the one of the most challenging group for pediatric oncologists (Fig. 2). Treatment consists of induction chemotherapy to achieve remission, local control with surgery and radiotherapy followed by consolidation of remission with ASCT oral 13-*cis*-retinoic acid (CRA), with or without antibody immunotherapy.

Dose-intensive induction chemotherapy

The goal of induction chemotherapy is rapid reduction of the entire tumor burden: both metastatic and primary sites, the latter to facilitate complete resection of soft tissue disease. Various combinations of cyclophosphamide, ifosfamide, doxorubicin, cisplatin, carboplatin, etoposide, topotecan and vincristine have been used with CR/VGPR rates of 50–80%.[41–45] Improved supportive care has facilitated the administration of dose-intensive regimens that may have improved response rates over the last two decades.

Surgery

Although most tumors respond to chemotherapy, surgery is critical to achieving complete remission (CR) in primary site for most patients, since radiotherapy alone is generally unable to sterilize soft tissue sites.[46] Gross total resection was correlated with reduced risk of local recurrence in retrospective analyses, especially when combined with dose-intensive induction chemotherapy and local radiotherapy.[47] For abdominal primaries, the thoracoabdominal approach allows better visualization of great vessels and may result in more complete resection.[48]

Radiotherapy

NB is considered to be a radiosensitive tumor and radiotherapy is a critical component of local control of the primary site where it is applied in the setting of minimal residual disease post-induction therapy and surgery. Radiotherapy has also been applied to metastatic sites of bulk disease after achieving a chemoresponse.[49] Current protocols usually employ a dose of 2100 cGy to the primary site as consolidation therapy via either fractionated or hyperfractionated regimens, though randomized trials have not been performed.[50] At MSKCC a combination of dose-intensive chemotherapy, surgery and hyperfractionated radiotherapy to the primary site at a dose of 2100 cGy resulted in a local relapse rate of <10%, a critical path to achieving long-term cure.[47]

Myeloablative therapy

Patients treated with modest doses of induction chemotherapy appeared to benefit from total body irradiation (TBI)-based myeloablative chemotherapy followed by ASCT using bone marrow or peripheral blood stem cells especially after the addition of CRA.[18] This led to ASCT (without TBI) being accepted by most investigators as key to improving survival. However, the role of additional myeloablative therapy to dose-intensive induction therapy has not been studied prospectively. Tandem, triple, allogeneic, and combined ^{131}I-metaiodobenzylguanidine (MIBG)/ASCT are being explored in clinical trials, some with encouraging results.[16,51–53] A new phase III COG study randomizes patients to single versus tandem transplants. At MSKCC, dose-intensive induction therapy was adopted since 1987, and myeloablative chemotherapy did not reduce the incidence of CNS or systemic relapse.[54]

Immunotherapy to maintain remission

GD2, a surface glycolipid antigen that is ubiquitous and abundant on neuroblastoma cells is an ideal target for immunotherapy.[55] Its expression on normal tissues is restricted to neurons which are protected from the effects of intravenous (IV) monoclonal antibodies (MoAbs) by the blood brain barrier.[56] The profound immunosuppression produced by high-dose chemotherapy regimens for NB, while creating unfavorable conditions for application of active immunotherapy, allows the use of passive immunotherapy after the completion of induction chemotherapy or ASCT without the development of human anti-mouse neutralizing antibody.[57,58] Anti-GD2 MoAbs currently form the mainstay of neuroblastoma immunotherapy and their safety profile has been well-established.[59,60] Acute toxicities include pain and allergic reactions. Long-term toxicities have not been encountered.

The murine IgG3 MoAb 3F8 initially developed in 1985 has undergone extensive preclinical testing and was the first MoAb to be studied in patients with NB.[61] In preclinical studies, 3F8 has the slowest dissociation rate among anti-GD2 antibodies[62] and mediates dose-dependent destruction of NB by human complement and by human lymphocytes,[63] cultured monocytes,[64] and granulocytes.[65] It utilizes both FcγRII and FcγRIII Fc receptors for neutrophil ADCC (antibody-dependent cell-mediated cytotoxicity) and the CR3 receptor for iC3b-mediated cytotoxicity.[66,67] Based on strong in vitro evidence showing the synergy between 3F8 and granulocyte–macrophage colony stimulating factor (GM-CSF),[65] a phase II study was carried out in patients with chemorefractory disease. It

demonstrated a CR rate of >80% by histology in the marrow and by MIBG scan.[68] When 3F8 was utilized to consolidate remission after multimodality therapy in patients with stage 4 NB diagnosed >12 months of age, a significant improvement in long-term EFS was observed when compared to historical controls.[59] In the most recent update of the clinical utility of 3F8, 164 patients with high-risk NB in first remission treated on consecutive 3F8 protocols from 1991 through 2006 were analyzed. At diagnosis, 147 patients were >18 months old with bone marrow and/or bone metastases. 45% had *MYCN*-amplified NB. All had standard dose-intensive induction therapy. Patients received either: (1) 3F8 (±targeted radiotherapy with ^{131}I-3F8) (ClinicalTrials.gov NCT00002634, NCT00040872) instead of ASCT or (2) 3F8 plus intravenous (iv) GM-CSF (ClinicalTrials.gov NCT00002560); or (3) 3F8 plus subcutaneous (sc) GM-CSF [ClinicalTrials.gov NCT00072358]. Long-term progression-free survival (PFS) among group 1 ($n = 42$, median followup of 13 years among survivors) was 43 ± 8%. In group 3 ($n = 64$, followup of 3 years), PFS improved to 73% ± 6% ($p = 0.015$) while for group 2 ($n = 58$, followup of 7 years), PFS was 53 ± 7%. ASCT prior to 3F8 + GM-CSF immunotherapy did not improve outcome.

Ch14.18 consists of the variable region of murine MoAb 14.18 and the constant regions of human IgG1-K.[69] It demonstrates ADCC and CDC (complement-dependent tumor cytotoxicity) of NB and melanoma cells in vivo.[70–72] Based on encouraging clinical responses in phase I studies, ch14.18 was tested in large phase II studies as consolidation therapy for stage 4 NB (German NB90 and NB97 studies). For the 166 patients >12 months at diagnosis EFS was similar in patients receiving ch14.18 when compared to patients on maintenance chemotherapy. However, OS was improved, and rate of bone marrow relapse reduced, in patients treated with ch14.18.[73] Recently early data from the COG randomized phase III trial of ch14.18 plus cytokines after ASCT (ClinicalTrials.gov NCT00026312) demonstrated a clear survival advantage in patients receiving immunotherapy when compared to untreated controls.[74]

Differentiation therapy with retinoids

In *vitro*, retinoids, vitamin A derivatives, induce differentiation and growth arrest of malignant NB cells probably through binding to retinoid acid receptors.[75] Intracellular retinol is metabolized to all-trans retinoic acid, which then activates a number of nuclear receptors that heterodimerize and regulate gene transcription.[76] CRA administered after myeloablative chemotherapy improved EFS in a randomized phase III trial and is accepted by most investigators for frontline therapy for patients in remission.[18] However, retinoid therapy has, in general, not been associated with responses in patients with measurable or evaluable disease.[77] Fenretinide, a newer retinoid was studied in a phase I COG clinical trial that established MTD (maximum tolerated dose).[78] However, the relatively poor bioavailability of fenretinide from ingested tablets has led to ongoing phase I trials utilizing newer oral formulations with improved bioavailability and intravenous formulation of fenretinide.

Treatment approaches for refractory or relapsed NB

Despite advances in treatment and improvement in survival for patients with high-risk NB over the last three decades, significant obstacles to achieving cure remain. Firstly, 20–50% of patients have soft tissue or osteomedullary NB that is refractory to induction chemotherapy. The advent of second line chemotherapy, immunotherapy and targeted therapies has improved survival for this group of patients, but cure remains out of reach for most. Secondly, a majority of patients who achieve remission relapse in bone/BM or less commonly, in soft tissue sites. Newer strategies using non-cross-resistant therapies are required to affect cures in this group. Thirdly, as it becomes clear that the CNS is a sanctuary site for NB in some patients, isolated CNS relapses are being detected in a small, though increasing number of patients. The prognosis for this group of patients was hitherto dismal,[79,80] but recent advances potentially can significantly improve survival in this group of patients.[81,82]

Treatment for relapsed or refractory soft tissue or osteomedullary disease

Several studies have focused on therapies for relapsed NB due to the relatively large number of patients who are resistant to standard therapies. With the advent of newer treatments and the use of increasingly sophisticated modalities to detect asymptomatic relapse, OS time after relapse has increased.[83,84] Currently open trials are summarized in Table 3 along with their Clinicaltrials.gov identifiers.

Second line chemotherapy

Over the last two decades several new chemotherapeutic agents with anti-NB activity have been studied. The camptothecins topotecan[85–87] and irinotecan[88,89] have proven anti-NB activity and have been extensively used in salvage regimens. Until recently the combination of cyclophosphamide and topotecan was the first line salvage regimen studied by the COG. With the incorporation of this combination into front line therapy in the current COG protocol for newly diagnosed high-risk NB, it is likely that a further well-studied combination: irinotecan plus temozolomide[90] will be increasingly utilized for resistant NB. Both combinations have demonstrated anti-NB utility though CR/VGPR are rare. At MSKCC, camptothecins are combined with high-dose cyclophosphamide both for anti-NB effect and to permit the administration of the murine antibody 3F8 for consolidation of response to chemotherapy.[58] Other new chemotherapeutic agents with potential anti-NB activity include ABT-751, an oral anti-tubulin agent, though no complete or partial responses were observed in the initial phase I study.[91]

MoAb mediated Immunotherapy

Unmodified antibodies

MoAb 3F8 in combination with GM-CSF has been shown to be highly effective against chemorefractory bone marrow NB,[92] with a histologic marrow CR response of >80%.[68] Similar response rates are unavailable for ch14.18 when used alone or in combination with cytokines. Results have been less impressive for patients with significant disease burden. Beta glucans (BG), complex carbohydrate polymers bind to CR3 and enhance iC3b-mediated cytotoxicity initiated by complement-activating antibodies such as 3F8.[93–95] In a phase I study barley-derive BG in combination with 3F8 led to objective responses in 40% of patients. Two patients developed immune thrombocytopenia as DLT (dose limiting toxicity) though MTD was not reached.[96] A phase I study of yeast derived BG + 3F8 is currently under way. At MSKCC current initiatives are focused on producing highly effective humanized forms of 3F8 as well as other humanized antibodies for other tumor antigens.

Immunocytokines

Immunocytokines can activate and redirect effectors to human tumors. The human interleukin 2 (rIL-2) molecule was linked to the COOH terminus of each human IgG1 heavy chain of ch14.18 to create the immunocytokine ch14.18-IL2 that retained the specificity and the effector function of ch14.18,[97] activated human effector cells,[98] and suppressed xenografts.[99] In an effort to further

Table 3
Currently open trials for refractory or relapsed stage 4 neuroblastoma.

Category	Agent/s	Phase	Clinicaltrials.gov identifier
NB-specific trials			
Antibody-based	3F8 + GM-CSF	II	NCT00072358
	Heat-modified 3F8 + GM-CSF	I	NCT00450307
	3F8 + yeast-derived beta glucan	I	NCT00492167
	^{131}I-3F8 + bevacizumab	I	NCT00450827
	^{131}I-3F8 intra-Ommaya	II	NCT00445965
	Humanized 14.18K322A	I	NCT00743496
	^{131}I-8H9 intra-Ommaya	I	NCT00089245
Vaccine	Whole cell vaccine modified to secrete IL2 and lymphotactin	I/Ii	NCT00703222
	Trivalent vaccine + adjuvants	I	NCT00911560
	Multivalent vaccine	I	NCT00944580
Anti-angiogenic	ZD6474 ± CRA	I	NCT00533169
Chemotherapy-based	Irinotecan + bortezomib	I	NCT00644696
	Topotecan, vincristine + doxorubicin	II	NCT00392340
	Melphalan + buthionine sulfoximine		NCT00005835
	ABT-751	II	NCT00436852
Cell-based	Chimeric receptor-transduced T-cells + anti-CD45 antibody	I	NCT00609206
	Chimeric receptor-transduced T-cells	I	NCT00085930
	Haploidentical NK cells + IL2	I	NCT00698009
	Haploidentical NK cells + 3F8 + GM-CSF	I	NCT00877110
Differentiating agents	Fenretinide (intravenous)	I	NCT00646230
	Fenretinide (oral lipid matrix)	I	NCT00295919
Other agents	Nifurtimox	I	NCT00486564
	Nifurtimox + cyclophosphamide + topotecan + zoledronic acid	II	NCT00601003
	Zoledronic acid + cyclophosphamide	I	NCT00206388
^{131}I-MIBG-based	^{131}I-MIBG (no-carrier added)	I/IIa	NCT00659984
	^{131}I-MIBG + carboplatin, etoposide, melphalan	II	NCT00253435
	^{131}I-MIBG + irinotecan and vincristine	I	NCT00509353
	^{131}I-MIBG + arsenic trioxide	II	NCT00107289
	^{131}I-MIBG + vorinostat	I	NCT01019850
Targeted small molecules	CEP-701	I	NCT00084422
Trials for pediatric tumors including NB			
Antibody-based	Ipilumumab (anti-CTLA4)	I	NCT00556881
	Lexatumumab (TRAIL receptor)	I	NCT00428272
Anti-angiogenic	Cediranib	I	NCT00354848
Chemotherapy	Pemetrexed	II	NCT00520936
	XK469 (quinoxalines analog)	I	NCT00028522
	Ixabepilone	I	NCT00030108
	Vinorelbine + cyclophosphamide	II	NCT00180947
	Gemcitabine + oxaliplatin	II	NCT00407433
Targeted small molecules	MLN 8237 (Aurora kinase inhibitor)	I/II	NCT00739427
	Dasatinib	I/II	NCT00788125
	Gefitinib + irinotecan	I	NCT00132158
	Perifosine	I	NCT00776867
	TPI287 ± temozolomide	I	NCT00867568
	PF-02341066 (ALK inhibitor)	I	NCT00939770
Cell-based	Tumor lysate pulsed dendritic cells + lymphocytes	Pilot	NCT00526240
	Tumor lysate pulsed dendritic cells	Pilot	NCT00405327
Other agents	Radiolabeled octreotide	I	NCT00049023
	Trabectadin	I	NCT00437047

reduce anti-mouse antibody responses, hu14.18-IL2 was produced and used in clinical trials.[100] Twenty-seven children with NB were treated in the phase I COG study with MTD of 12 mg/m^2/day. DLTs included prolonged neutropenia, anaphylaxis, hyperbilirubinemia and hypotension and half life was 3.3 h. In the recently concluded phase II study, preliminary response rate of 21% for patients bone marrow disease was reported while patients with bulky disease did not respond.[101]

Radiolabeled antibodies

Due to its radiosensitivity, NB is an attractive tumor for radioimmunotherapy (RIT). RIT has the potential to target radiation to metastatic sites while avoiding the toxicities of external beam radiation which can be severe in young children. ^{131}I-3F8 targets selectively to NB primary tumors and metastatic sites in lymph nodes, BM, and bone with superior sensitivity when compared to ^{131}I-MIBG.[102] Safety was initially established in a phase I study in which a dose of 28 mCi/kg was reached without MTD being reached. Toxicities included self-limited pain, fever and rash, followed by myelosuppression that required BM rescue. Other than hypothyroidism, no extramedullary toxicity was observed. ^{131}I-3F8 at a dose of 20 mCi/kg followed by autologous BMT was added to a multimodality program for high-risk NB patients (n = 35): the MSKCC N7 protocol. With continued followup (6–10 years from diagnosis), overall survival for NB patients newly diagnosed at >18 months of age is ~40%.[103,104] Preclinical studies indicate that

the combination of targeted radiotherapy and anti-angiogenesis effectively suppressed NB xenografts even at relatively low doses of ^{131}I-3F8.[105] A clinical trial based on these observations is currently underway at MSKCC in which ^{131}I-3F8 is dose-escalated while the dose of the anti-angiogenic agent bevacizumab is kept constant. DLTs have not been encountered at the first two dose levels.[106] ^{131}I-ChCE7, a MoAb that targets an L1 isoform has been used for successful radioimmunodetection of NB with sensitivity and specificity superior to ^{131}I-MIBG.[107]

Adoptive cell therapy

Dendritic cells

NB derived gangliosides have been shown to inhibit dendritic cell differentiation and function, and may play a role in tumor-induced immunosuppression and tumor escape from surveillance.[108,109] Several methods of producing functional dendritic cells with anti-NB activity have been described in children.[110,111] In two reported phase I studies using autologous dendritic cells pulsed with autologous tumor cell RNA or cell lysate in children with stage 4 NB safety and tumor-specific humoral immune responses in some patients were described but objective responses were not observed.[112,113]

Natural killer (NK) cells

NK cells demonstrate anti-NB activity via several mechanisms: (a) NK cells bear activating receptors whose ligands are expressed on NB (b) they express CD16 a receptor required for binding MoAb (e.g., 3F8 or Ch14.18) and triggering NK-mediated cytotoxicity.[114–116] Among children with high-risk NB undergoing autologous stem-cell transplantation plus 3F8 immunotherapy, improved overall and progression-free survival are associated with the absence of one or more HLA class I ligands for the patient's NK cell inhibitory killer inhibitory receptor (KIR).[117] These results suggest that NK tolerance is modified after ASCT, and that KIR-HLA genotypes may influence MoAb-based immunotherapy.

T-cells

The anti-NB activity of T-cells is limited by the low expression of HLA antigens on NB. T-cells can be retargeted using antibody-based chimeric receptors to overcome this and early clinical investigations using these engineered T-cells have demonstrated elicitation of anti-NB immune responses.[118,119]

Vaccines

Several preclinical approaches have been tested including whole cell vaccines, GD2 mimics, anti-idiotypes, DNA and peptide injection. Whole cell vaccines engineered to express multiple transgenic immunostimulatory molecules can stimulate the immune system. In patients, NB cell lines and autologous NB tumor transduced with cytokines have elicited immune responses and have not been associated with major side effects.[120–122] A1G4, the anti-idiotypic MoAb for 3F8 was the first anti-idiotypic antibody for neuroblastoma to go into clinical trial. In a phase I study of children with relapsed NB or high-risk GD-positive solid tumors at MSKCC, A1G4 was administered intravenously at 0.1, 0.3 and 1 mg/kg for a total of 10 doses. There were no DLTs. Anti-GD2 antibody responses were detected at all dose levels[55] (Cheung et al., unpublished data). 1A7, another anti-idiotypic MoAb[123] directed against 14G2a was administered in conjunction with the adjuvant QS-21 patients with high-risk NB in CR or VGPR. Treatment was well tolerated, typically with local reactions. Anti-1A7 responses were observed in all patients. Encouraging long-term survival was observed in patients treated in first CR, 17 of 20 patients had no evidence of disease 47 months from study entry. However, only 1 of 11 patients in subsequent CR/VGPR survived.[124] Current clinical vaccine trials include modifications of whole cell vaccine approaches and GD2 mimics conjugated to newer more potent immune stimulators.[125,126]

^{131}I-MIBG therapy

MIBG, a guanethidine derivative, is taken up >90% NB tumors by both active and passive mechanisms*. ^{131}I labeled MIBG (^{131}I-MIBG) has been used to target radiation for the therapy of metastatic NB for the last three decades. Treatment is well tolerated, common side effects limited to myelosuppression (often necessitating stem-cell support),[127] biochemical hypothyroidism and transient sialoadenitis.[81] ^{131}I-MIBG monotherapy achieves responses in 18–37% of refractory or relapsed patients, usually at doses $\geqslant$12 mCi/kg, though responses are usually transient. Dose-escalating ^{131}I-MIBG therapy beyond 18 mCi/kg by administering two doses did not improve response rates.[128] The addition of high-dose chemotherapy to ^{131}I-MIBG therapy resulted in increased toxicity without improving efficacy.[129–132] Current studies are investigating the role of radiosensitizers such as irinotecan, topotecan, and arsenic trioxide in possibly enhancing the anti-NB activity of ^{131}I-MIBG. A no-carrier added form of ^{131}I-MIBG that has the potential to enhance targeting of radiation is being tested in a phase I study.

Targeted therapies

Anaplastic lymphoma kinase (ALK)

ALK is a receptor tyrosine kinase implicated in the genesis of several malignancies including lymphoma and infantile myofibroblastic tumors possibly by modifying the responsiveness of the mitogen-activated protein kinase pathway to growth factors. ALK kinase is constitutively activated by gene amplification at the ALK locus in several NB cell lines, though *ALK* amplification is rarely observed in NB tumor samples.[133,134] In a recent study, *ALK* was identified as a familial NB predisposition gene.[135] Activating mutations or rearrangements can also be somatically acquired in 8–16% of sporadic NB cases.[136] Screening NB cell lines with pharmacological antagonists of the ALK kinase domain has identified ALK as a molecular target.[137] ALK inhibitors are currently being tested for therapy of anaplastic large cell lymphoma and may potentially benefit a subset of NB patients.

TrkB

The neurotrophin receptor TrkB is preferentially expressed in aggressive NB tumors and the BDNF/TrkB signaling pathway have been shown to form an autocrine loop in these tumors.[138,139] Several components of the pathway including Trk tyrosine kinases, PI-3-kinase, Akt and its downstream members can be targeted by small molecule inhibitors. A drug targeting Trk tyrosine kinases (CEP-751) has shown preclinical efficacy against NB mouse xenografts,[140] and is currently in a NB clinical trial.

Insulin-like growth factor-1 (IGF-1)

IGF-1 regulates growth of NB cells via AKT and MAP kinase pathways.[141] IGF-1 receptor antagonists have been proven to have anti-NB activity in xenograft models.[142,143] Unlike sarcomas, it is not clear if IGF-1 receptor is overexpressed on NB cells. Nevertheless, NB patients have been included in ongoing phase I studies of IGF-1 receptor inhibitors.

p53 pathway

p53 gene mutations are rare in NB at diagnosis.[144,145] Chemotherapy-induced apoptosis in MYCN-amplified tumors may be p53 dependent.[146] However, p53 inactivation via mutation or

MDM2 activation is often observed in relapsed tumors and in NB cell lines and is associated with drug resistance.[147,148] Reactivation of the p53 pathway, e.g., with nutlin 3 that inhibits MDM2 may reverse drug resistance[149] and may have a role in therapy of relapsed neuroblastoma. Selective checkpoint kinase (e.g., Chk1) inhibitors may also have utility in enhancing the efficacy of DNA-damaging agents especially when the p53 pathway is defective.[150] One of the mechanisms of anti-NB activity of HDAC inhibitors in vitro is the restoration of the p53 pathway in NB cells lines.[151] Anti-NB activity has also been demonstrated in NB xenograft models.[152,153] The FDA approval of HDAC inhibitors for other malignancies may permit their rapid testing for patients with resistant NB.

Angiogenesis

High-risk NB tumors show evidence of increased tumor angiogenesis with increased microvessel density. This pro-angiogenic phenotype is promoted by growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) transforming growth factor alpha (TGF-alpha) and platelet-derived growth factor A (PDGF-A). An association between increased levels of the matrix metalloproteinases MMP-2 and -9 and advanced tumor stage has also been observed. The integrins alpha(v)beta3 and alpha(v)beta5 – markers of angiogenic endothelium – were also found to be more highly expressed in blood vessels of high-risk NB.[154] Several drugs have shown anti-angiogenic activity in preclinical NB models; these include chemotherapeutic agents such as vinblastine and topotecan, retinoids[155] and thalidomide.[156] Specific anti-VEGF strategies tested include the anti-VEGF humanized monoclonal-antibody bevacizumab,[157] and VEGF-TRAP.[157] In a recent completed phase I trial of bevacizumab in children, treatment was well tolerated. However, no objective responses were found.[158] Ongoing phase I studies are testing other anti-angiogenic molecules in children with NB. It is unlikely that these molecules will have efficacy as single agents; combination with chemotherapy or radiotherapy will likely be necessary for clinical responses.

Other therapies

In preclinical studies, zoledronic acid appears to have anti-NB activity on bony metastases by inhibiting osteoclasts as well as direct suppression of tumor cell proliferation.[159] It is currently being tested in a phase I study.

The importance of PI3K/Akt pathway in maintaining NB cell growth[141,160] has led to interest in examining possible anti-NB activity of inhibitors of this pathway in the clinic. Several drugs including chemotherapeutic agents inhibit PI3K/Akt but preliminary clinical data on specific inhibitors such as rapamycin or temsirolimus used as single agents are disappointing. Perifosine, a synthetic alkyphospholipid accumulates in cell membrane and disrupts PI3K/Akt and MAP kinase pathways and is currently being studies in phase I studies in patients with NB.[161]

Treatment of CNS relapse

The prognosis for NB patients experiencing isolated CNS relapse has been grim with median survival of 5.3 months.[79,80] A recent treatment strategy that utilizes intrathecal RIT as part of a multimodality regimen has shown great promise and may radically improve prognosis for patients with relapsed CNS NB.[81,82] 17/21 patients treated with a combination of surgical resection of CNS parenchymal disease, craniospinal radiotherapy, chemotherapy with irinotecan and temozolomide and RIT with ^{131}I-3F8 or ^{131}I-8H9 survive 7–74 months (median 33 months) after isolated CNS relapse.[80] MoAb 8H9 is a murine IgG1 against cell surface antigen 4Ig-B7H3, which is present on many solid tumors, but restricted on normal tissues particularly normal CNS tissues.[162] In an ongoing phase I study of ^{131}I-8H9, DLT was not encountered at treatment doses from 10 to 70 mCi. Targeting of leptomeningeal disease was demonstrated by ^{124}I-8H9 scans.[163] Calculated mean radiation dose to the cerebrospinal fluid (CSF) was 36.3 (range 12.8–106) cGy/mCi; mean blood dose was 2.5 cGy/mCi.

Conclusions and future directions

The divergent clinical presentations and outcomes of this relatively rare pediatric tumor have fascinated pediatric oncologists caring for patients with NB over the last several decades. The improved understanding of NB biology has provided a strong rationale for risk group stratifications where cytotoxic therapy can be reduced or eliminated for about 30–40% of children with NB, e.g., those with locoregional/4S disease, thus avoiding the long-term side effects of such therapy. However, major challenges still remain for children diagnosed with stage 4 NB at >18 months of age or those whose tumors are *MYCN*-amplified. While modern dose-intensive chemotherapy, aggressive surgery and local radiotherapy succeed in obtaining CR/VGPR, cure rates are still <35% for most treatment centers. Refractory soft tissue disease (e.g., retroperitoneal, liver, and lung), although less common than resistant osteomedullary metastases, is often harder to cure. The typical scenario of osteomedullary relapse despite achieving CR/VGPR, demands urgent attention given to the biology and treatment of minimal residual disease. MoAb 3F8 appears to be effective for chemorefractory osteomedullary disease and is associated with prolonged remission both at MSKCC and in neuroblastoma treatment centers in Hong Kong (Godfrey Chan et al., 2009, personal communication). With the initiation of a phase III randomized study, 3F8 may become more widely available and be more effectively utilized. With improved systemic control and prolonged survival, relapses at sanctuary sites have become the next hurdle. A promising multimodality regimen employing MoAbs has brought new hope for patients with CNS metastases once considered to be uniformly lethal.

Young children with NB are reaching toxicity limits from dose-escalation of chemotherapy.[41,45,16,164] While high-dose chemotherapy is important in achieving remissions, long-term side effects including secondary leukemia and organ failures are high prices to pay. As OS improves, more chronic issues related to cytotoxic therapies, e.g., hearing deficit, delayed growth and developmental problems[19,165] are not uncommon. Although novel drugs or small molecules directed at specific pathways or targets will be found, it is unlikely they will change the outlook of neuroblastoma as single agents. A better understanding of the interplay between pharmacogenomics, tumor and its microenvironment, is critical. A discipline to integrate and exploit all these different modalities in the appropriate clinical context is essential in order to achieve the ultimate endpoint, i.e., curing patients and improving their quality of life.

Conflict of interest statement

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