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Neuroblastoma

Executive Summary

Neuroblastoma is the most common extracranial tumour of childhood and the third most common pediatric cancer. The age standardized incidence in North America is 8.9–12.5 per million with a slightly higher incidence in males. The median age at diagnosis is 18 months, with the highest incidence in infants aged less than 6 months. Diagnosis is very rare after age 10 years. The most common sites of primary tumours are the thorax and abdomen. Approximately 50% of patients present with metastases, most commonly to the lymph nodes, bone or bone marrow.

Patients with neuroblastoma can be classified into 3 risk categories: low, intermediate and high risk based on clinical (age and stage) and biologic (molecular and genetic) factors. Low risk cases most commonly include localized tumours that can be observed or surgically resected and rarely require chemotherapy treatment. Intermediate risk tumours are biologically favourable unresectable large tumours that cross the midline and often invade the spinal cord, vessels or organs. These patients often receive moderate doses of chemotherapy followed by surgical resection. In contrast, most high risk patients have metastatic disease and require intensive treatment with chemotherapy, radiation, surgery, autologous stem cell transplant (since the mid- to late 1990s) and, more recently, immunotherapy. Five year overall survival rates for low and intermediate risk patients are more than 90%, while rates for high risk patients are less than 50%.

For patients treated in Ontario during the study period, data collected in the Pediatric Oncology Group of Ontario Networked Information System (POGONIS) included stage, age and treatment modalities (chemotherapy, stem cell transplant). A total of 435 cases of neuroblastoma were diagnosed in Ontario from 1985 to 2004 with an average of 21 cases per year. Interestingly, the incidence of neuroblastoma increased 9.5% from the 1985–1989 period to the 2000–2004 period. The increase was most significant for infants less than 1 year of age with stage 1 disease and was most likely due to increased detection of small, low risk tumours. The numbers of patients diagnosed with stage 2, 3 and 4 disease was essentially stable during 1985–2004.

Overall survival was 64.6% for all patients with neuroblastoma in Ontario during the 20 year study period, improving from 58.6% in 1985–1989 to 73.5% in 2000–2004. Event free survival (EFS), for which data were collected only during the latter 10 years of the study, improved from 58.1% (1995–1999) to 68.6% (2000–2004). Age and stage are 2 highly prognostic factors. Notably, 2 of 115 patients with localized neuroblastoma (stage 1 and 2) died of their disease. Among patients with metastatic (stage 4) neuroblastoma over 18 months of age in the most recent period (2000–2004), the 5 year EFS and overall survival were 39.3% and 48.5%, respectively. These rates represent an improvement of 170% since the 1985–1989 period. Since the mid-1990s, autologous bone marrow transplants (BMT) or stem cell transplants have become the standard of care – the use of BMT increased from 78.0% (1995–1999) to 90.9% (2000–2004).

More than one third of patients with neuroblastoma experience 1 or more relapses. The proportion of patients whose disease recurred decreased from 41.0% (1995–1999) to 26.4% (2000–2004). The majority of relapses occurred in patients with stage 4 disease. Of the total recurrences, 83.3% and 77.7% were stage 4 at initial diagnosis in the 1995–1999 and 2000–2004 periods, respectively. The incidence of recurrence may decrease further in the post-2004 periods as a result of increased use of immunotherapy with anti-GD2 antibodies and cytokines. Five year overall survival post relapse is only 20%, which is similar to outcomes reported for other registries. The improvements in survival post relapse may be due to increased use of chemotherapies and other treatments. Increasing use of targeted inhibitors and high dose metaiodobenzylguanidine (MIBG) therapy for relapsed neuroblastoma may lead to improved survival rates, response duration or both in future cohorts of patients.

Introduction

Neuroblastoma is the most common extracranial pediatric solid tumour and the most frequently diagnosed cancer in infants. Neuroblastoma includes a spectrum of neural crest tumours that range from benign, self-resolving tumours to disseminated malignant tumours that account for more deaths than any other pediatric solid tumour.

Epidemiology

The incidence of neuroblastoma varies significantly in different countries, with frequency generally associated with the medical resources available. Lower frequency of diagnosis has been observed in countries with fewer medical resources. In North America and Europe, national incidences have been estimated at between 8.9 and 12.5 cases per million population.[1–4] Most studies suggest a slight male predominance, similar to other common childhood solid tumours. Notably, a 1997 review from the Canadian National Cancer Incidence Reporting System reported a higher incidence in males (11.0 vs. 9.6 per million).[2]

Neuroblastoma is primarily a disease of infants and toddlers, with a median age at diagnosis of approximately 18 months. The reported age-specific incidence rates for infants less than 6 months of age range from 52.3 to 72.5 cases per million. In children aged 1–4 years, the incidence falls to 18.1–19.5 cases per million. The incidence continues to fall with increasing age to approximately 1 per million in children aged 10–14 years.[1]

Clinical Presentation

Presenting symptoms vary depending on the location of the primary tumour, which may occur anywhere along the course of the sympathetic chain from the brain to the pelvis, and on the extent of the disease. Many symptoms are a direct result of local effects of the tumour mass on organs, vessels or nerves; symptoms occasionally may be secondary to a paraneoplastic process. The primary tumour site for more than 65% of patients is the abdomen, most commonly the adrenal gland. Related symptoms can include hypertension, abdominal pain and constipation. In infants thoracic tumours are more common and symptoms may include Horner syndrome (unilateral ptosis, anhydrosis and myosis) and respiratory symptoms.[5–7] Spinal cord compression presenting as paraplegia and loss of bladder function can result from centrally-located paraspinal tumours. In more than 50% of patients, neuroblastoma metastasizes to bone, bone marrow, lymph nodes or the liver, and less commonly, in infants, to skin. Although uncommon, metastases to the orbital bone result in peri-orbital bruising, proptosis and potentially visual impairment. Metastases to the liver in infants can result in hepatomegaly and secondary renal failure and respiratory compromise. Metastases to the lung and brain are rare at diagnosis, but central nervous system disease at relapse is increasingly common.[5–7]

Diagnosis and Staging

Many investigations are required to confirm the diagnosis of neuroblastoma and evaluate disease extent. In most cases, tumour tissue is required to obtain pathologic, biologic and molecular information to determine prognosis and choice of therapy. The choice between biopsy or upfront resection depends on patient characteristics and, most importantly, the presence of metastatic disease. Diagnosis of neuroblastoma can be confirmed either by histologic diagnosis on biopsy or resection specimen, or by elevated urinary catecholamines together with tumour cells detected in the bone marrow.

Urinary catecholamines

Neuroblastomas are characterized by abnormal catecholamine synthesis resulting in increased levels of metabolites, including homovanillic acid (HVA) and vanillylmandelic acid (VMA). Elevated urinary HVA and VMA can be detected in approximately 90% of patients and provides a non-invasive, inexpensive, rapid method for disease detection and surveillance.[8-10] Most laboratories can now analyze levels in "spot" samples of approximately 10 mL.

Imaging assessment

As part of initial assessment of disease burden and assessment of surgical risks and resectability, patients require imaging of their primary tumour site and potential sites of metastases. Cross-sectional imaging modalities such as computed tomography and magnetic resonance imaging (MRI) are used to assess the primary tumour; lymph node involvement; and local invasion of vessels, nerves and organs at diagnosis, during therapy and for surveillance following completion of therapy. Urgent MRI is required if intraspinal invasion and cord compression are suspected.

Nuclear medicine scans are routinely used to identify metastatic disease. Technetium-99m-MDP bone scans have been used to detect cortical bony metastases of many types of solid tumours, including neuroblastoma. Since the late 1990s, however, technetium bone scans have been slowly replaced by scans using radiolabelled MIBG, a norepinephrine analogue specific to neural crest tumours. Of neuroblastomas, 90% are MIBG-avid and MIBG can identify soft tissue, bone and bone marrow metastases. Scoring systems (e.g., the Curie score) are now being incorporated to quantitate disease at diagnosis and during treatment.[11] In the 10% of patients with non-MIBG-avid disease, bone scans have been used for metastatic evaluation; however, evidence suggests that positron emission tomography (PET) provides more sensitive detection of metastatic bone and soft tissue lesions. Routine use of PET scans in these rare MIBG-negative patients will likely be recommended in the next set of international response criteria guidelines.[12,13]

Bone marrow

Advanced neuroblastoma typically metastasizes to bone marrow. Bone marrow aspirations and biopsies from at least 2 sites are required for staging. In certain cases in which bone marrow evaluation is positive, bone marrow neuroblasts may provide sufficient material for diagnosis and can be used to determine molecular and genetic markers (e.g., *MYCN* amplification; see below).

Upfront surgical resection

For localized, non-metastatic lesions, upfront surgery is often the approach of choice.[14] Factors influencing the resectability of the lesion include its size, location and relation to vital structures. Surgical resection alone may be the optimal form of therapy for patients with low stage disease. Any involved or potentially involved lymph nodes are resected or sampled at the time of surgery for staging.

Recent data from the International Neuroblastoma Risk Group (INRG) and the International Society of Paediatric Oncology Europe Neuroblastoma (SIOPEN) have demonstrated that a system to standardize image defined risk factors (IDRFs), including direct organ involvement, invasion of spinal cord and vascular encasement, can be used pre-operatively to determine which subsets of patients should have pre-operative chemotherapy.[15,16]

Tumour biopsy

Although upfront surgical resection is usually preferred for localized disease without IDRFs, many children will present with metastatic disease or large, unresectable lesions with IDRFs. In these cases, biopsy is performed to confirm the histologic diagnosis and to acquire tissue to test for specific molecular and genetic characteristics. Although traditionally most biopsies were performed by surgical incision ("open"), more recently at some institutions biopsies have been obtained by laparoscopy or by image guided needle core biopsies. However, it is critical that sufficient biopsy tissue be obtained to test molecular and genetic markers required for risk stratification (see below).[17]

Prognostic Factors and Risk Stratification

Large cooperative group studies and international databases have resulted in the identification of statistically robust clinical and biologic (molecular and genetic) factors that predict prognosis and can be used to define clinical risk groups to tailor the intensity of adjuvant therapy. For patients treated in Ontario during the study period, the main risk factors for which data were collected included International Neuroblastoma Staging System (INSS),[18] stage and age. The biologic predictors were identified over the timeframe of this sample and were either not known or not collected throughout most of the timeframe covered, and thus are not available in the current POGONIS dataset.

Factors Determining Risk Assignment

Age

Age has long been recognized as an important predictor of outcome in neuroblastoma.[19] Historically, age of less than 12 months was considered a favourable prognostic factor, and compared with patients older than 12 months these infants were often treated with less intensive therapy. More recently, large retrospective studies have demonstrated that age is a continuous variable and that the cutoff for favourable prognosis could be extended to age 18 months for the subset of patients with biologically favourable tumours.[20–22]

Stage

A variety of systems have been used internationally to stage disease in patients with neuroblastoma. In an attempt to facilitate comparison of results across various international cooperative groups, a consensus system, the INSS, was first published in 1988 and updated in 1993; it has been used for the POGONIS data (Appendix 1).[23,18]

INSS staging is by definition post surgical. It can vary depending on surgical experience and aggressiveness. Stages 1 and 2 represent completely and incompletely resected tumours with or without local lymph node involvement, respectively. Stage 3 tumours cross the midline and stage 4 involves distant metastases. Stage 4s (4 special) represents a distinct subset of metastatic neuroblastoma, defined as presentation at less than 12 months of age and with metastatic spread limited to skin, liver and/or bone marrow, but not bone. Although these patients usually have biologically favourable tumours, infants with stage 4s disease, especially those less than 2 months of age, experience significant morbidity and mortality due to hepatomegaly, infection and respiratory and renal failure.[24,25]

A newer, pre-surgical staging system, the International Neuroblastoma Risk Group Staging System (INRGSS), was developed in Europe to stage tumours based on IDRFs, independent of whether the tumour crosses the midline, its size or individual surgical preferences.[26] The INRGSS is being prospectively studied in North American cooperative group trials.

Histology

In 1984, Shimada et al. published an age-based prognostic pathologic classification system of neuroblastic tumours that factored in Schwannian stromal proportion and the mitosis-karyorrhexis index, a surrogate marker for proliferation.[27] The International Neuroblastoma Pathology Classification (INPC) updated the original Shimada classification to incorporate other prognostic histologic factors, including mitotic rate, calcification and differentiation.[28,29]

MYCN amplification

Increased copies (more than 10) of the *MYCN* oncogene is the most powerful biologic predictor of poor outcome.[30] Approximately 20% of all neuroblastoma tumours demonstrate *MYCN* amplification (MYCNA); the incidence is higher (40%) in stage 4 patients. Several studies in North America and Europe have demonstrated that even for infants *MYCN* status can discriminate recurrence risk. For example, the Children's Cancer Group (CCG) reported that infants with stage 4 disease had 3 year EFS of 93% in the absence of *MYCN* amplification, compared with 10% for those with *MYCN* amplification tumours.[31] Although in older children (over 18 months) with metastatic disease *MYCN* amplification is not an independent predictor of long term outcome, in studies of patients with INSS stage 2 or 3 disease *MYCN* status may predict prognosis.[32,33]

The current standard technique for detecting *MYCN* amplification is fluorescence in situ hybridization (FISH). Genetic techniques that can simultaneously detect gain and loss at multiple genetic loci may soon replace FISH, however.

DNA ploidy

Ploidy, or tumour DNA content (chromosome number), is a powerful predictor of relapse free survival and is especially informative for the subset of patients younger than 18 months or patients with low stage disease. Hyperdiploid tumours (DNA index greater than 1) with an increased amount of DNA compared with diploid tumours (DNA index = 1) as determined by flow cytometry are associated with a more favourable prognosis.[34,35]

Other chromosomal alterations

Over the past decade the presence of segmental chromosome aberrations (small losses and gains of chromosomal material), including allelic losses at 1p and 11q and additional chromosomal gains and losses, have been shown to predict poor prognosis.[36,37] Based on these studies, current and future clinical trials are likely to include the presence of segmental aberrations (as detected by FISH or newer technologies such as array comparative genomic hybridization and single nucleotide polymorphism analyses) as a prognostic factor to inform risk group assignment and tailor therapies.

Contemporary data suggest that for children older than 18 months at diagnosis who present with metastatic disease (stage 4), additional tumour biomarkers are not independently prognostic. In contrast, for children younger than 18 months with metastatic disease, and for patients who present with localized disease, the identification of specific biologic and clinical risk factors has enabled more precise prediction of prognosis and determination of the most appropriate treatment intensity.

Risk Groups and Treatment

Currently the majority of North American pediatric oncology centres use the Children's Oncology Group (COG) criteria to establish risk groups (Appendix 2). The COG risk stratification uses age, stage, *MYCN* status, tumour ploidy and INPC histologic classification. In future studies the system will likely include the status of specific chromosomal losses and gains. Currently, in North America centralized laboratories supported by the COG and the U.S. National Cancer Institute perform some of these studies for patients on clinical trials. However, a systematic approach to the development of Ontario's capacity and funding to perform and interpret these assays needs to be developed.

Low risk disease

The majority of patients with low risk disease have localized tumours (INSS stage 1 or 2) with favourable biology. Patients with low risk disease may receive only initial diagnostic or definitive surgery, or both, with no chemotherapy. Furthermore, over the past decade some studies have supported observation only for small, biologically favourable tumours, which often spontaneously regress or differentiate. Asymptomatic infants with small tumours detected incidentally, such as on prenatal and neonatal ultrasounds, are followed with frequent imaging studies (optimally ultrasounds) and urinary catecholamine measurement to confirm resolution over time. This approach may allow a high percentage of infants to avoid surgery and surgical morbidity.[38-40]

Low risk patients who are symptomatic (i.e., with spinal cord compression or large hepatomegaly) often require chemotherapy to relieve their symptoms. These approaches have recently been reported to result in EFS of 87% and overall survival of 96%.[14]

Intermediate risk disease

The majority of intermediate risk neuroblastoma patients are characterized by unresectable large masses (usually INSS stage 3). Over the past decade the intermediate risk group has expanded to include INSS stage 4 patients younger than 18 months with favourable biology, a group of patients previously treated as high risk. The identification of a diverse group at intermediate risk, defined in multiple ways and treated with multiple chemotherapy regimens, has resulted in excellent reported survival rates (greater than 75–80%) by a variety of cooperative groups.[41,42] The COG recently reported that overall survival of above 90% could be maintained by using a risk-stratified approach to therapy that reduces chemotherapy for patients with biologically favourable

tumours.[43] Based on these results, current strategies are aimed at reducing intensity and duration of chemotherapy in the majority of patients with more favourable intermediate risk disease, while identifying clinical and biologic characteristics of intermediate risk patients at the highest risk for relapse in an effort to maintain excellent survival while reducing short and long term toxicities.

High risk disease

In contrast to the excellent survival associated with low risk and intermediate risk neuroblastoma, EFS for high risk disease remains poor. For patients treated in the 1990s and most of the 2000–2010 period, EFS for high risk disease was approximately 40%.[44-48]

Therapy for high risk disease involves 3 phases of treatment: induction, consolidation and maintenance. For induction, patients receive 5–7 cycles of dose intense chemotherapy and undergo surgical resection of the primary tumour (usually after cycle 4 or 5); 85–90% of patients initially respond to this chemotherapy. However, there are both acute and long term toxicities, including infections, multi-organ dysfunction and hearing and renal morbidities.

Following induction therapy, current protocols for high risk disease include consolidation therapy with high dose myeloablative chemotherapy followed by autologous BMT or stem cell transplant. Transplants for neuroblastoma patients were initially performed using harvested bone marrow cells. Over the past decade autologous peripheral blood stem cells have been routinely used to repopulate the marrow. In contrast to bone marrow and peripheral blood stem cell transplants for hematopoietic malignancies, for neuroblastoma stem cells are re-infused as a rescue to enable the use of high dose myeloablative chemotherapy.

The use of autologous stem cell transplant or rescue has consistently shown short and long term survival advantages. A prospective randomized clinical trial by the CCG confirmed the improved long term survival for stem cell transplant compared with chemotherapy (5 year EFS, 30% vs. 19%).[46,47] Similar results have been reported from the German Paediatric Oncology and Haematology Society (GPOH).[45,49] No advantage has been demonstrated for methods that attempt to purge contaminating neuroblastoma cells from the peripheral blood stem cells.[48] Current and future trials will be aimed at improving the efficacy of consolidation regimens with increased intensity, such as double autologous transplants, or modifications in conditioning strategies.

Despite intensive induction chemotherapy and consolidation with BMT or peripheral blood stem cell transplant, recurrence rates remained high, with more than 60% of patients experiencing relapse, presumably owing to the presence of clinically undetectable minimum residual disease. Oral cis-retinoic acid (cis-RA) administered following transplant consolidation therapy has been shown to significantly improve outcomes and is now the standard of care for maintenance therapy.[46]

During the past 10 years additional agents for high risk patients in the post consolidation or maintenance phases of therapy have been examined, including immunotherapies that target the disialoganglioside GD2 that is almost universally expressed on the surface of neuroblastoma cells. A COG phase 3 randomized controlled trial demonstrated that the addition of anti-GD2 chimeric (human-mouse) Ch14.18, interleukin-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) to cis-RA produced outcomes superior to that of cis-RA alone (2 year EFS of 66% vs. 46%).[48] Similar long term data from the GPOH demonstrated improved EFS almost 10 years following administration of Ch14.18 post transplant.[50] Immunotherapy with Ch14.18, interleukin-2 and GM-CSF is now considered the standard of care for all high risk neuroblastoma patients as part of their upfront post consolidation treatment. The production of Ch14.18 in North America will move from the U.S. National Cancer Institute to a commercial pharmaceutical company and the cost will not be covered by clinical trials once commercially licensed. Thus it will be critical to obtain national regulatory approvals and provincial funding for the approximately 8–10 high risk patients treated annually with immunotherapy in Ontario.

Relapsed disease

Despite the recent treatment advances for high risk neuroblastoma, relapsed or refractory disease is often fatal.[51,52] If relapse is localized, surgery, radiation therapy or both can be curative; however, for patients with recurrent metastatic disease, particularly following upfront intensive high risk therapy, current salvage therapies can prolong survival but cure only a minority of patients.

Importantly, a variety of second line therapies palliate symptoms and prolong life for many years.[53-56] Several retrospective studies have reported the survival for patients following relapse or progression of neuroblastoma. The largest analysis (of 8,800 patients from the INRG) reported that the 5 year overall survival for the 2,266 patients who relapsed between 1990 and 2002 was 20%.[57] Agents in clinical trials include immunotherapies and tyrosine kinase inhibitors, including inhibitors of the anaplastic lymphoma kinase, which is mutated in 10% of sporadic neuroblastoma tumours.[58] Targeted radiotherapy with high dose MIBG has shown a response rate of 37% in the refractory disease setting and in 2014 is available to patients in Ontario.[59]

Future Directions

With contemporary therapy, approximately 50% of patients with high risk neuroblastoma still experience recurrence and those that are cured have significant late effects. With traditional chemotherapeutic agents now approaching maximal intensity, new targeted therapies are required. One such strategy will involve the addition of high dose MIBG therapy to upfront treatment for subsets of high risk neuroblastoma patients. Approaches to improve the efficacy and decrease the toxicity of immunotherapy are also active areas of investigation. In contrast, for patients with low or intermediate risk neuroblastoma, recent North American and European trials support continued reduction in chemotherapy for subsets of patients based on favourable clinical and biologic risk factors.

For all patients with neuroblastoma it is expected that there will be integration of additional molecular and genetic features to further refine risk stratification. For example, specific signatures of gene expression in tumours can predict outcome among patients with high risk neuroblastoma to distinguish an ultra high risk group with EFS below 20%.[60,61] Whole-genome sequencing strategies will likely help identify novel tumour targets as well as host (patient) genetic sequences that predict the development of toxicities to specific therapies, such as hearing loss.[62] These advances will lead to further personalized or precision medicine for patients with neuroblastoma.

Data Collection

The slow but steady improvement in neuroblastoma survival in the past 3 decades is the result of sequential clinical trials performed by cooperative groups such as the COG in North America, the SIOPEN and the GPOH. Clinical trial participation requires patient or family consent and results therefore may not truly represent population outcomes, since many patients do not enter studies because of parental choice, severity of presenting disease, eligibility or timing criteria and study availability.

POGONIS provides an opportunity to capture trends in incidence and outcome of neuroblastoma on a population basis, independent of clinical trial participation. This population-based sample allows assessment of the translation of successful clinical trials to real world results in a population. One caveat specifically related to outcomes for the neuroblastoma population, however, is that information on many of the contemporary prognostic factors and biomarkers used to stratify therapy in recent international cooperative group studies was either not available during the period under consideration or POGONIS did not collect it, thus limiting some of the potential comparisons. Nevertheless, the presence of stage and age data allows for basic risk stratification and comparison of trends and outcomes for many of the relevant risk groups with larger cohorts of patients in Ontario.

Discussion

EXHIBIT 9.1: **Incidence of primary neuroblastoma by age at diagnosis and year of diagnosis, age 0–14, in Ontario, 1985–2004**

		All years 1985–2004	1985–1989	1990–1994	1995–1999	2000–2004
Stage	Age group at time of diagnosis	N	N	N	N	N
All	Overall	**435**	**95**	**118**	**118**	**104**
	< 1 year	154	34	40	38	42
	1–4 years	185	38	55	49	43
	≥ 5 years	96	23	23	31	19
1	Overall	**58**	**8**	**15**	**15**	**20**
	< 1 year	32	4	5	10	13
	1–4 years	19	3	8	2	6
	≥ 5 years	7	1	2	3	1
2	Overall	**64**	**14**	**19**	**17**	**14**
	< 1 year	35	8	11	9	7
	1–4 years	23	5	8	5	5
	≥ 5 years	6	1	0	3	2
3	Overall	**87**	**21**	**25**	**23**	**18**
	< 1 year	38	8	10	11	9
	1–4 years	34	8	12	7	7
	≥ 5 years	15	5	3	5	2
4	Overall	**192**	**42**	**46**	**61**	**43**
	< 1 year	23	7	4	7	5
	1–4 years	107	22	25	35	25
	≥ 5 years	62	13	17	19	13
4s	Overall	**27**	**7**	**11**	**1**	**8**
	< 1 year	26	7	10	1	8
	1–4 years	1	0	1	0	0
	≥ 5 years	0	0	0	0	0
Missing stage	Overall	**7**	**3**	**2**	**1**	**1**

Exhibit 9.1

There were 435 cases of neuroblastoma diagnosed in Ontario from 1985 to 2004. The average number of cases per year was 22. The overall number of neuroblastoma cases has increased at a rate comparable to the increase in total population in Ontario. The absolute number of cases per 5 year period increased 9.5% from 1985–1989 to 2000–2004. This difference was caused primarily by the marked (150%) increase in stage 1 disease, particularly among children less than 1 year of age. A greater than 3-fold increase in incidence was detected for infants with stage 1 disease diagnosed in the most recent period (2000–2004) compared with the earliest period (1985–1989). This increase may be attributed to increased recognition of low stage asymptomatic masses incidentally detected by imaging investigations ordered to investigate unrelated symptoms, such as chest x-ray for cough or abdominal ultrasound for urinary tract infection. In addition, the increased use of prenatal ultrasounds has likely contributed to the detection of adrenal masses in the third trimester.

The number of stage 2, 3, 4 and 4s cases remained essentially stable over all 4 study periods. Approximately half of all cases involved infants or toddlers aged less than 18 months, a proportion similar to that of other groups, including a report of the Italian Registry from a similar period (1979–2005): 45% of that study's 2,216 patients were younger than 18 months.[63] It should be noted that while many of these incidentally detected localized neuroblastoma masses were historically treated by surgical resection, chemotherapy or both during the periods under study, more recent studies support observation for a subset of these patients with small adrenal masses.[40] Current COG and SIOPEN trials are also investigating the role of any intervention in a subset of larger localized masses with favourable biology in children under 18 months.[64]

EXHIBIT 9.2: **5 year overall survival with primary neuroblastoma tumours by stage, age of diagnosis and year of diagnosis, age 0–14 years, in Ontario, 1985–2004**

		All years 1985–2004			1985–1989		
Stage	Age group at time of diagnosis	N	Overall survival	%	N	Overall survival	%
All	Overall	**421**	**272**	**64.61**	**87**	**51**	**58.62**
	0–28 days	21	17	80.95	4	3	75.00
	0–12 months	151	139	92.05	34	29	85.29
	12–18 months	41	25	60.98	10	6	60.00
	18+ months	229	108	47.16	43	16	37.21
1	Overall	**53**	**52**	**98.11**	**7**	**7**	**100.00**
	0–28 days	5	5	100.00	1	1	100.00
	0–12 months	30	30	100.00	4	4	100.00
	12–18 months	4	4	100.00	0	0	—
	18+ months	19	18	94.74	3	3	100.00
2	Overall	**62**	**61**	**98.39**	**12**	**12**	**100.00**
	0–28 days	2	2	100.00	0	0	—
	0–12 months	35	35	100.00	8	8	100.00
	12–18 months	5	5	100.00	1	1	100.00
	18+ months	22	21	95.45	3	3	100.00
3	Overall	**84**	**68**	**80.95**	**20**	**15**	**75.00**
	0–28 days	3	3	100.00	0	0	—
	0–12 months	37	35	94.59	8	7	87.50
	12–18 months	11	8	72.73	4	4	100.00
	18+ months	36	25	69.44	8	4	50.00
4	Overall	**190**	**64**	**33.68**	**40**	**11**	**27.50**
	0–28 days	3	1	33.33	0	0	—
	0–12 months	23	17	73.91	7	5	71.43
	12–18 months	19	7	36.84	5	1	20.00
	18+ months	148	40	27.03	28	5	17.86
4s	Overall	**27**	**23**	**85.19**	**7**	**5**	**71.43**
	0–28 days	8	6	75.00	3	2	66.67
	0–12 months	26	22	84.62	7	5	71.43
	12–18 months	1	1	100.00	0	0	—
	18+ months	0	0	—	0	0	—
Missing stage	Overall	**5**	**4**	**80.00**	**1**	**1**	**100.00**

	1990–1994			1995–1999			2000–2004			1995–2004		
	N	Overall survival	%	N	Overall survival	%	N	Overall survival	%	N	Overall survival	%
Total	**115**	**72**	**62.61**	**117**	**74**	**63.25**	**102**	**75**	**73.53**	**219**	**149**	**68.04**
Eye	5	4	80.00	3	3	100.00	9	7	77.78	12	10	83.33
Head and neck	38	36	94.74	38	37	97.37	41	37	90.24	79	74	93.67
Thorax	18	12	66.67	5	4	80.00	8	3	37.50	13	7	53.85
Abdomen	59	24	40.68	74	33	44.59	53	35	66.04	127	68	53.54
Stage 1	**12**	**12**	**100.00**	**15**	**14**	**93.33**	**19**	**19**	**100.00**	**34**	**33**	**97.06**
Eye	0	0	—	0	0	—	4	4	100.00	4	4	100.00
Head and neck	3	3	100.00	10	10	100.00	13	13	100.00	23	23	100.00
Thorax	3	3	100.00	0	0	—	1	1	100.00	1	1	100.00
Abdomen	6	6	100.00	5	4	80.00	5	5	100.00	10	9	90.00
Stage 2	**19**	**18**	**94.74**	**17**	**17**	**100.00**	**14**	**14**	**100.00**	**31**	**31**	**100.00**
Eye	1	1	100.00	0	0	—	1	1	100.00	1	1	100.00
Head and neck	11	11	100.00	9	9	100.00	7	7	100.00	16	16	100.00
Thorax	3	3	100.00	1	1	100.00	0	0	—	1	1	100.00
Abdomen	5	4	80.00	7	7	100.00	7	7	100.00	14	14	100.00
Stage 3	**25**	**19**	**76.00**	**22**	**20**	**90.91**	**17**	**14**	**82.35**	**39**	**34**	**87.18**
Eye	0	0	—	2	2	100.00	1	1	100.00	3	3	100.00
Head and neck	10	10	100.00	11	11	100.00	8	7	87.50	19	18	94.74
Thorax	5	3	60.00				2	1	50.00	2	1	50.00
Abdomen	10	6	60.00	11	9	81.82	7	6	85.71	18	15	83.33
Stage 4	**46**	**11**	**23.91**	**61**	**21**	**34.43**	**43**	**21**	**48.84**	**104**	**42**	**40.38**
Eye	1	0	0	1	1	100.00	1	0	0	2	1	50.00
Head and neck	4	2	50.00	7	6	85.71	5	4	80.00	12	10	83.33
Thorax	5	2	40.00	4	3	75.00	5	1	20.00	9	4	44.44
Abdomen	37	7	18.92	50	12	24.00	33	16	48.48	83	28	33.73
Stage 4S	**11**	**11**	**100.00**	**1**	**1**	**100.00**	**8**	**6**	**75.00**	**9**	**7**	**77.78**
Eye	3	3	100.00	0	0	—	2	1	50.00	2	1	50.00
Head and neck	10	10	100.00	1	1	100.00	8	6	75.00	9	7	77.78
Thorax	1	1	100.00	0	0	—	0	0	—	0	0	—
Abdomen	0	0	—	0	0	—	0	0	—	0	0	—
Unstaged	**2**	**1**	**50.00**	**1**	**1**	**100.00**	**1**	**1**	**100.00**	**2**	**2**	**100.00**

Exhibit 9.2

Overall survival

Survival data are available for all periods from 1985 to 2004. The cohort comprised 421 patients whose 5 year overall survival during that period was 64.6%. There is evidence of improved outcome with time. The 5 year overall survival for 2000–2004 was 73.5%, compared with 58.6% in the earliest period (1985–1989).

Localized disease

Similar to those in published international cohorts, patients recorded in POGONIS with INSS stage 1 or 2 disease had excellent overall survival in all time periods, with only a single death in each stage group reported for the entire review period.[14,26] This excellent survival rate justifies the development of trials aimed at decreasing treatment for this group to lessen exposure to chemotherapies and surgery and thus decrease the incidence of acute and long term effects.

Metastatic disease

The most striking improvements in outcome were observed for stage 4 patients. The 5 year overall survival for stage 4 patients improved almost 2-fold, from 27.5% in 1985–1999 to 48.8% in 2000–2004. The improvement in survival was most pronounced in stage 4 patients older than 18 months (overall survival 17.9% vs. 48.5%).

Age and biomarkers in metastatic neuroblastoma

Age is a well-known prognostic factor in neuroblastoma. Specifically, the outcome for patients older than 18 months is significantly worse than that for those younger than 18 months.[65] The overall survival for Ontario patients over 18 months improved by 77.5% in the most recent period compared with 1985–1989. The use of myeloablative chemotherapy with autologous BMT and more recently stem cell transplant became the standard of care starting in the latter part of the 1990s[45,46] and thus we hypothesize that this improvement in survival is due to adoption of protocols in Ontario that include consolidation with bone marrow or stem cell transplant. In support of this hypothesis, the percentage of stage 4 patients older than 18 months who underwent transplant in Ontario increased from 78.0% (1995–1999) to 90.9% (2000–2004) (data not shown).

Although data on the use of BMT are not available before 1995, the majority of protocols did not include BMT as the standard approach in the early 1990s. The most recent COG study for high risk neuroblastoma conducted between 2001 and 2006 included 6 cycles of induction chemotherapy, surgery and autologous stem cell transplant following high dose chemotherapy with cyclophosphamide, etoposide and melphalan. The 5 year overall survival and EFS were 50% and 38%, respectively.[45]

The 5 year overall survival for stage 4 patients older than 18 months in Ontario in 2000–2004 was similar to the 50% survival rate for the COG study (study A3973) that was open during this period.[45] That study enrolled patients who met criteria for high risk disease (other than stage 4 patients who were over 18 months); thus, 14% of patients were under 18 months owing to the presence of unfavourable biology (usually *MYCN* amplification) and 14% were stage 3 (because of *MYCN* amplification, unfavourable histology or both). Since biomarker data are not available we can compare only the entire stage 4 population over age 18 months in Ontario with the overall A3973 cohort of 495 patients.

Historically all patients with stage 4 disease were treated as high risk and received similar treatment. Data from the late 1990s demonstrated that infants (under 12 months) with stage 4 disease in the absence of *MYCN* amplification had a significantly better outcome and thus, most centres in North America (including Ontario) have been treating these patients with intermediate risk protocols, which include moderate dose chemotherapy and no stem cell transplant. In a CCG study, the EFS for stage 4 infants with *MYCN* amplification treated with intermediate risk therapy was 10%, compared with 93% for those without *MYCN* amplification. In Ontario the 5 year overall survival for stage 4 patients younger than 12 months was above 80% for the period 1995–2004. This suggests that the majority of these patients had favourable biology and specifically were less likely to have had tumours with *MYCN* amplification. COG study A3961, which enrolled patients with intermediate risk neuroblastoma between 1997 and 2005, included 176 infants with stage 4 non-*MYCN* amplification disease. These patients received moderate dose intermediate risk therapy; their EFS and overall survival were 81% and 93%, respectively.[43]

EXHIBIT 9.3: **5 year event free survival with primary neuroblastoma tumours by stage, age at diagnosis and year of diagnosis, age 0–14 years, in Ontario, 1995–2004**

		All years: 1995–2004			1995–1999			2000–2004		
Stage	Age group at time of diagnosis	N	Event free survival	%	N	Event free survival	%	N	Event free survival	%
All	**Overall**	**219**	**138**	**63.01**	**117**	**68**	**58.12**	**102**	**70**	**68.63**
	0–28 days	12	9	75.00	3	3	100.00	9	6	66.67
	0–12 months	79	69	87.34	38	34	89.47	41	35	85.37
	12–18 months	13	7	53.85	5	4	80.00	8	3	37.50
	18+ months	127	62	48.82	74	30	40.54	53	32	60.38
1	**Overall**	**34**	**32**	**94.12**	**15**	**14**	**93.33**	**19**	**18**	**94.74**
	0–28 days	4	3	75.00	0	0	0	4	3	75.00
	0–12 months	23	22	95.65	10	10	100.00	13	12	92.31
	12–18 months	1	1	100.00	0	0	—	1	1	100.00
	18+ months	10	9	90.00	5	4	80.00	5	5	100.00
2	**Overall**	**31**	**30**	**96.77**	**17**	**17**	**100.00**	**14**	**13**	**92.86**
	0–28 days	1	1	100.00	0	0	0	1	1	100.00
	0–12 months	16	14	87.50	9	9	100.00	7	6	85.71
	12–18 months	1	1	100.00	1	1	100.00	—	—	—
	18+ months	14	14	100.00	7	7	100.00	7	7	100.00
3	**Overall**	**39**	**31**	**79.49**	**22**	**17**	**77.27**	**17**	**14**	**82.35**
	0–28 days	3	3	100.00	2	2	100.00	1	1	100.00
	0–12 months	19	16	84.21	11	9	81.82	8	7	87.50
	12–18 months	2	1	50.00	0	0	—	2	1	50.00
	18+ months	18	14	77.78	11	8	72.73	7	6	85.71
4	**Overall**	**104**	**37**	**35.58**	**61**	**19**	**31.15**	**43**	**18**	**41.86**
	0–28 days	2	1	50.00	1	1	100.00	1	—	0.00
	0–12 months	12	10	83.33	7	6	85.71	5	4	80.00
	12–18 months	9	4	44.44	4	3	75.00	5	1	20.00
	18+ months	83	23	27.71	50	10	20.00	33	13	39.39
4s	**Overall**	**9**	**7**	**77.78**	**1**	**1**	**100.00**	**8**	**6**	**75.00**
	0–28 days	2	1	50.00	0	0	—	2	1	50.00
	0–12 months	9	7	77.78	1	1	100.00	8	6	75.00
	12–18 months	0	0	—	0	0	—	0	0	—
	18+ months	0	0	—	0	0	—	0	0	—
Missing stage	**Overall**	**2**	**2**	**100.00**	**1**	**1**	**100.00**	**1**	**1**	**100.00**

Exhibit 9.3

The POGONIS data for the most recent 10-year period (1995–2004) allow for the calculation of EFS in addition to overall survival. The 5 year EFS for all patients in that period was 63.0%, with superior outcomes noted in the most recent 5 year period (68.6% vs. 58.1%). The INRG database includes clinical outcome, stage, age and biomarker data for 8,800 patients diagnosed with neuroblastoma between 1990 and 2002 from Europe, North America and Japan.[66] The 5 year EFS and overall survival in the INRG cohort were 63% and 70%, respectively, which is very similar to the POGONIS data for this period. The majority of events (55) were recurrences. The Italian Neuroblastoma Registry reported that only 10% of deaths were due to toxicity, surgical complications or second malignancies.[63]

Relapsed disease

Relapse data have been collected in POGONIS for the 2 most recent 5 year periods (1995–1999 and 2000–2004). As expected based on the improved survival rates, the relapse rates were lower in 2000–2004 than in 1995–1999. Of 118 patients, 48 (40.6%) experienced at least one relapse of neuroblastoma in 1995–1999, compared with 27 of 104 (25.9%) in 2000–2004. As expected, the majority of relapses occurred in the higher stages (3 and 4, non-4s). Stage 4 patients accounted for 83.3% of the recurrences in 1995–1999 and 77.7% in 2000–2004 (data not shown). Our data are very similar to those published by the INRG, in which 2,266 of 8,800 (25.7%) patients experienced recurrence and 72% had stage 4 disease.[57] Similarly, in the Italian Registry 40% of 2,216 patients developed a relapse or progression.[64] The patients studied in these publications, much like those covered by the POGONIS data, were treated pre-2005 and thus the majority would have been unlikely to have received immunotherapy. The addition of anti-GD2 antibody (Ch14.18), interleukin-2 and GM-CSF in the post transplant setting is likely to improve the relapse free survival of high risk patients.[48]

EXHIBIT 9.4a: **1 year overall survival after relapse**

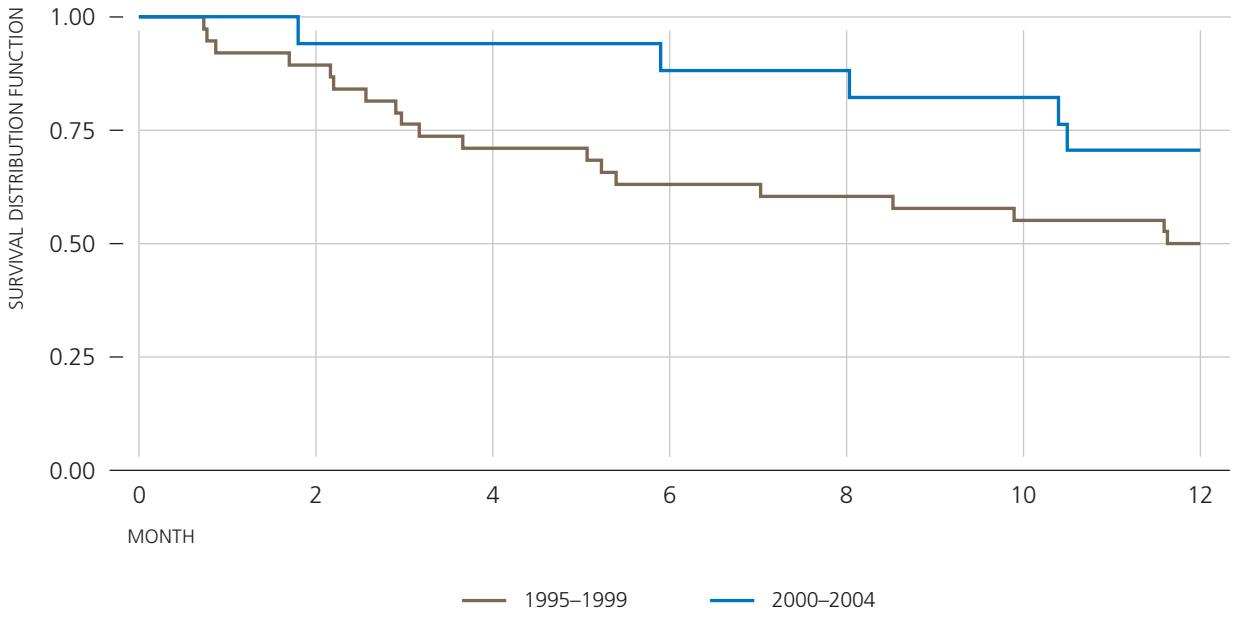

EXHIBIT 9.4b: **3 year overall survival after relapse**

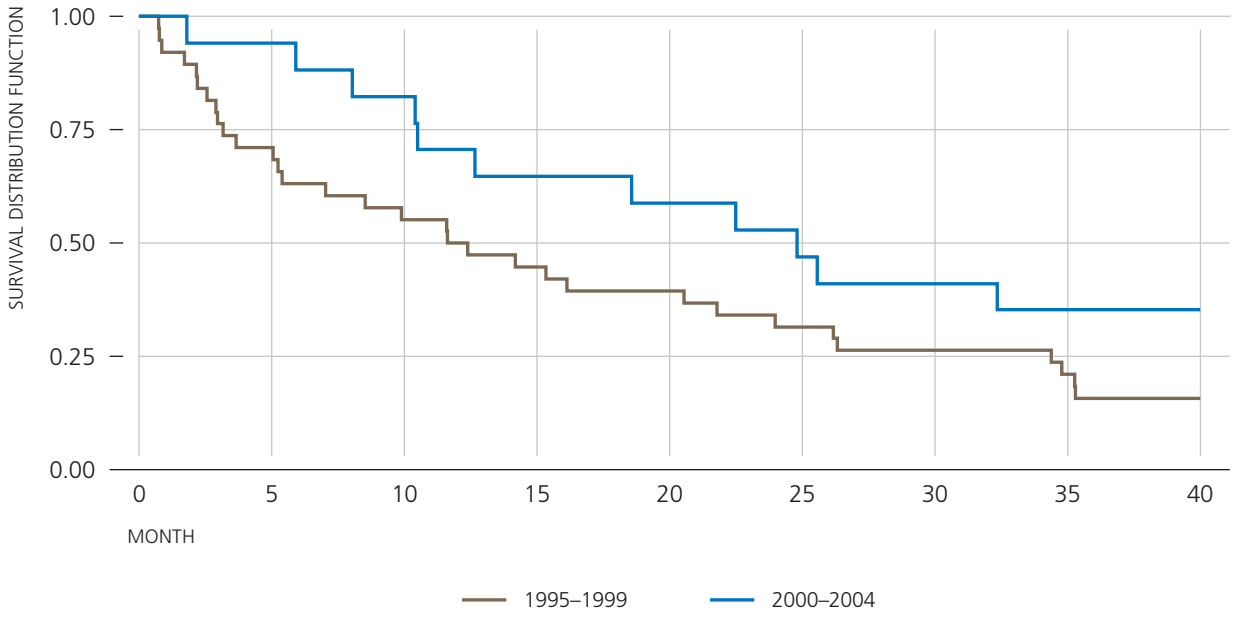

EXHIBIT 9.4c: **5 year overall survival after relapse**

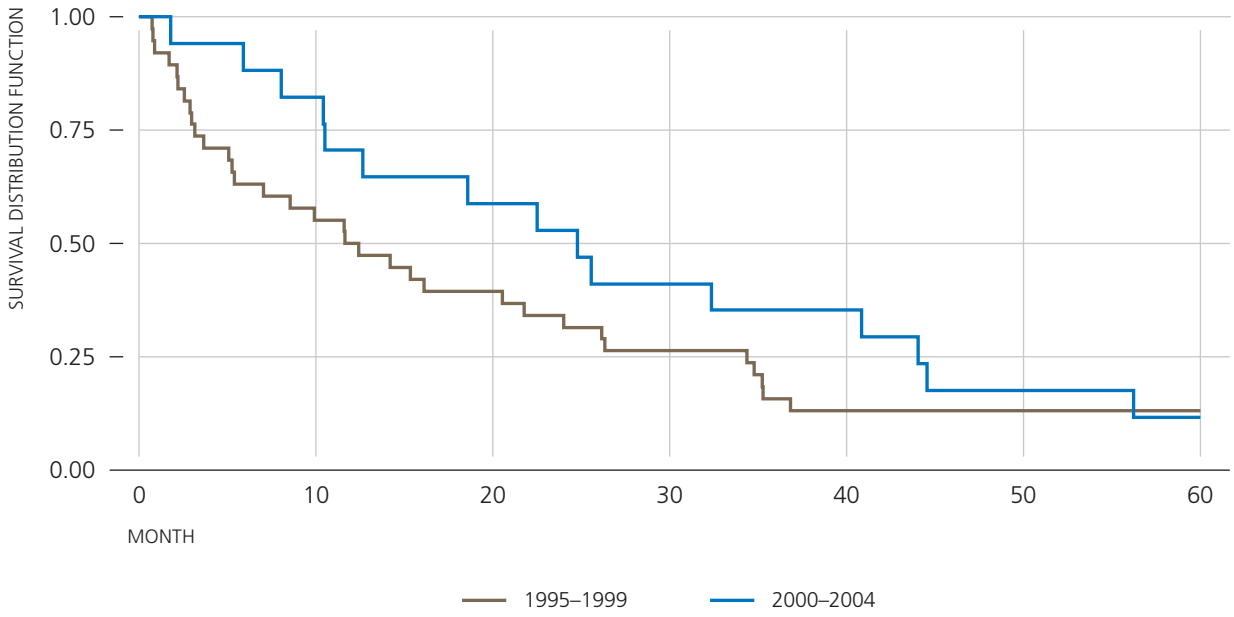

Exhibit 9.4a–9.4c

Survival post relapse

There were fewer relapses in 2000–2004 than in 1995–1999 (data not shown). For those patients who experienced a relapse, the overall survival at 1, 3 and 5 years post relapse was much higher in the 2000–2004 period, suggesting improvements in salvage therapy and illustrating that a significant portion of relapsed patients live for many years following relapse. This supposition is supported by the data from INRG, which show that patients with relapsed neuroblastoma had a 5 year overall survival of 20%, though outcome was significantly worse for patients who had initially presented with metastatic disease.[57] Increasing use of targeted inhibitors and high dose MIBG therapy may result in improved salvage rates and prolonged survival for patients with recurrent neuroblastoma.

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Appendices

APPENDIX 1: **International Neuroblastoma Staging System (INSS)[23]**

Stage 1	Localized tumour with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumour microscopically (nodes attached to and removed with the primary tumour may be positive).
Stage 2a	Localized tumour with incomplete gross resection; representative ipsilateral non-adherent lymph nodes negative for tumour microscopically.
Stage 2b	Localized tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour; enlarged contralateral lymph nodes must be negative microscopically.
Stage 3	Unresectable unilateral tumour infiltrating across the midline, with or without regional lymph node involvement, or localized unilateral tumour with contralateral regional lymph node involvement, or midline tumour with bilateral extension by infiltration (unresectable) or by lymph node involvement.
Stage 4	Any primary tumour 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 tumour (as defined for stage 1, 2a or 2b) with dissemination limited to skin, liver and/or bone marrow (limited to infants < 1 year of age).

APPENDIX 2: **Children's Oncology Group Risk Group and Protocol Assignment Schema[67]**

INSS stage	Age	MYCN status	Shimada histology	DNA ploidy	Risk group
1	0–21 yr	Any[a]	Any[b]	Any[c]	Low
2A/2B	<365 d	Any[a]	Any[b]	Any[c]	Low
	>365 d–21 yr	Non-amp	Any[b]	—	Low
	>365 d–21 yr	Amp	Fav	—	Low
	>365 d–21 yr	Amp	Unfav	—	High
3	<365 d	Non-amp	Any[b]	Any[c]	Intermediate
	<365 d	Amp	Any[b]	Any[c]	High
	>365 d–21 yr	Non-amp	Fav	—	Intermediate
	>365 d–21 yr	Non-amp	Unfav	—	High
	>365 d–21 yr	Amp	Any	—	High
4	<365 d	Non-amp	Any[b]	Any[c]	Intermediate
	<365 d	Amp	Any	Any	High
	>365 d–21 yr	Any	Any	—	High
4S	<365 d	Non-amp	Fav	>1	Low
	<365 d	Non-amp	Any[b]	=1	Intermediate
	<365 d	Non-amp	Unfav	Any[c]	Intermediate
	<365 d	Amp	Any	Any	High

Biology defined by MYCN status: amplified (Amp) vs. non-amplified (non-amp); Shimada histopathology: favourable (Fav) vs. unfavourable (Unfav); DNA ploidy: DNA index (DI) >1 or = 1; hypodiploid tumours (with DI <1) will be treated as a tumour with a DI >1.
[a]Must be "not amplified" or "amplified" cannot be unsatisfactory.
[b]Must be "favourable" or "unfavourable" cannot be inadequate.
[c]Must be >1 or = 1 (for patients <365 d) cannot be unsatisfactory.

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