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REDUCED RISK OF SECONDARY LEUKEMIA WITH FEWER CYCLES OF DOSE-INTENSIVE INDUCTION CHEMOTHERAPY IN PATIENTS WITH NEUROBLASTOMA

Brian H. Kushner, M.D.¹, Kim Kramer, M.D.², Shakeel Modak, M.D.³, Li-Xuan Qin, Ph.D.⁴, Karima Yataghena, M.D.⁵, Suresh C. Jhanwar, Ph.D.⁶, and Nai-Kong V. Cheung, M.D., Ph.D.⁷

¹Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. Tel: 212-639-6793. Fax: 212-717-3239. kushnerb@mskcc.org

²Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. Tel: 212-639-6410. Fax: 212-717-3239. kramerk@mskcc.org

³Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. Tel: 212-639-7623. Fax: 212-717-3695. modaks@mskcc.org

⁴Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, E. 63rd Street, New York, NY 10065, Tel: 646-735-8116; Fax: 646-735-0010; qinl@mskcc.org

⁵Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. Tel: 212-639-6140. Fax: 212-717-3492. yataghek@mskcc.org

⁶Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. Tel: 212-639-8895. Fax: 212-794-5830. jhanwars@mskcc.org

⁷Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. Tel: 646-888-2313. Fax: 212-744-2245. cheungn@mskcc.org

Abstract

Background—We report a prospective study of secondary leukemia (SL)/myelodysplastic syndrome (MDS) in neuroblastoma (NB) patients treated with 5 cycles of dose-intensive chemotherapy.

Procedure—NB patients received induction with high-dose cyclophosphamide (4200 mg/m²)-doxorubicin (75 mg/m²)-vincristine (cycles 1, 2, 4, 6, 8), and high-dose cisplatin (200 mg/m²)-etoposide (600 mg/m²) (cycles 3, 5, 7). Bone marrow was examined every 1–3 months for 36 months, with inclusion of extensive chromosomal studies 1–3 months post-induction and 1–2×/year thereafter.

Results—184 patients received 5 (n=76), 6 (n=45), 7 (n=59), or 8 (n=4) cycles. Eight patients developed SL/MDS (only one each in the 5- and 6-cycles groups), at 12–50 months, including two cases detected in surveillance studies. Among 108 patients who received 6 cycles, the 5-year cumulative incidence was 7.1% (95% CI: 2%, 12.2%), versus 0% among 54 patients who received

5 cycles without maintenance oral etoposide. Five-year cumulative incidences were 1.46%, 2.28%, and 8.47% among patients in the 5-, 6-, and 7-cycle groups, with fewer cycles having a significantly lower risk ($p=0.048$). There was no significant association of risk with potentially leukemogenic consolidative treatments (targeted radiotherapy, myeloablative therapy, and oral etoposide).

Conclusions—Reducing the number of dose-intensive cycles significantly decreases the risk of SL/MDS, yielding 5-year rates matching the low range (0.4% to 2.2%) reported for moderate-dose combination chemotherapy regimens used against other pediatric solid tumors.

Keywords

alkylating agents; chromosomal aberrations; topoisomerase II inhibitors

INTRODUCTION

Treatment for high-risk neuroblastoma (NB) at Memorial Sloan-Kettering Cancer Center (MSKCC)¹ and in recent French,² British,³ and Children's Oncology Group (COG)⁴ studies has included dose-intensive induction using alkylating agents, platinum compounds, and topoisomerase II inhibitors, as well as local radiotherapy (RT) that encompasses extensive areas of bone marrow (BM).^{5,6} Some multi-modality treatment programs for other poor-prognosis pediatric solid tumors also use dose-intensive chemotherapy. Pilot studies in the 1990s showed that this aggressive approach yielded excellent response rates but also ~10% risk of secondary leukemia (SL)/myelodysplastic syndrome (MDS).^{7–10} In contrast, the SL/MDS risk was very low (0.4% to 2.2%) with the standard moderate-dose therapy used for pediatric solid tumors at various times during the last 20 years.^{10–18}

With the aggressive dose-intensive approach as well as with the moderate-dose regimens, the two well-described kinds of SL/MDS occurred, namely, those associated with topoisomerase-II inhibitors and marked by early emergence of SL with translocations of the *MLL* gene at chromosome band 11q23,¹⁹ and those associated with alkylating agents and marked by an MDS prodrome, whole or partial deletions of chromosomes 5 or 7, and a latency period of 2–8 years.²⁰

Investigations into host risk factors may ultimately provide insights into how prone a given patient is to the leukemogenic effects of cytotoxic therapy. At present, however, minimizing exposure to leukemogens is the sole practical strategy for reducing the risk. We now report data validating that strategy in NB patients. The experience reflects a longstanding prospective approach initiated in 1990 and prompted by 1) concerns about SL/MDS among MSKCC Hodgkin's disease patients,²¹ and 2) a commitment to collect comprehensive toxicity data on the then novel MSKCC dose-intensive chemotherapy protocol for NB.¹ That protocol has been widely used in recent years.^{2–4}

PATIENTS AND METHODS

This analysis involved all high-risk NB patients diagnosed 1990–2006 and treated at MSKCC <8 months from the start of induction chemotherapy that consisted of 5 cycles of

a dose-intensive regimen: high-dose cyclophosphamide (4200 mg/m²)-doxorubicin (75 mg/m²)-vincristine (CAV) in cycles 1, 2, 4, 6, and 8, and high-dose cisplatin (200 mg/m²)-etoposide (600 mg/m²) (P/E) in cycles 3, 5, and 7 (Table I).^{1,4} The study cohort of 184 patients included 53 (all treated with 7 cycles) described previously.^{1,8} The number of cycles and post-induction treatments were determined by protocol. Cycles began when the absolute neutrophil count was >500/ μ l and the platelet count was >75,000–100,000/ μ l. Granulocyte colony-stimulating factor (G-CSF) was used after 1994.²² The cardioprotectant dexrazoxane, which is a topoisomerase II inhibitor and may be leukemogenic,²³ was not used. Informed written consents following institutional review board rules preceded all treatments and tests.

BM aspirates and biopsies were obtained and examined histologically at least every three months through ~36 months from the start of chemotherapy. BM karyotype and fluorescence *in-situ* hybridization (FISH) studies were done at diagnosis, 1–2 months after induction, 2–3 months after myeloablative therapy and stem-cell rescue, and once or twice annually thereafter. The FISH studies were performed looking for chromosomal aberrations associated with SL/MDS. Leukemias were classified morphologically by French-American-British criteria.²⁴

The cumulative incidence of SL/MDS was estimated using the method of competing risk.²⁵ Follow-up was counted from the start of induction chemotherapy to the development of clinical SL/MDS, detection of progressive NB, or last contact, whichever came first. NB progression was treated as the competing risk. Patients who died all had either leukemia or NB progression in the data. The equality of the cumulative incidence was compared across groups using the Gray's test.²⁶

RESULTS

Clinical characteristics (Table II)

The 184 patients (male:female, 1.3:1) had a median age at diagnosis of 3.3 years. Additional treatments included biologicals (13-*cis*-retinoic acid¹⁴ and anti-G_{D2} 3F8 monoclonal antibody²⁷) in 93% of patients, and RT (2100 cGy⁵ or 2160 cGy⁶) to the primary tumor site in 95% of patients. The predominant post-induction systemic treatments with leukemogenic potential in each group were: targeted RT using ¹³¹I-labeled 3F8²⁷ in the 7-cycle group; myeloablative therapy (MAT)^{28,29} in the 6-cycle group; and MAT^{28,29} and oral etoposide³⁰ in the 5-cycle group. The median follow-up for censored patients (date of NB progression or last contact) was 56.4 months.

Cases of SL/MDS (Table III): Group comparisons and clinical details

SL/MDS developed in 8 patients (only 1 each in the 5- and 6-cycles groups) at 12–50 months (median, 17). For the 108 patients who received 6 cycles, the 5-year cumulative incidence of SL/MDS was 7.1% (95% CI: 2%, 12.2%), compared to 0% among the 54 patients who received 5 cycles without oral etoposide, and 4.55% (95% C.I. 0%, 13.5%) among the 22 patients who received 5 cycles with oral etoposide. Cumulative incidences of SL/MDS at 5 years were 1.46% (95% C.I. 0%, 4.32%), 2.28% 95% C.I. 0%, 6.75%), and

8.47% (95% C.I. 1.26%, 15.69%) among patients in the 5-, 6-, and 7-cycle groups, with fewer cycles having a significantly lower risk ($p=0.048$; Figure 1). Subset analyses showed no significant association between SL/MDS and treatment with ^{131}I -3F8 in the 7-cycle group ($p=0.974$) or in the entire cohort of 184 patients ($p=0.343$); between SL/MDS and MAT in the 5-cycle group (0.345), in the 6-cycle group ($p=0.591$), or in the entire cohort of 184 patients ($p=0.338$). Among the 22 patients who received 5 cycles plus oral etoposide, one developed SL/MDS ($p=0.148$).

Among the patients who received 7 cycles ($n=59$) or 8 cycles ($n=4$), six developed SL/MDS (Table III). Three cases of MDS presented with thrombocytopenia 24 months from the start of induction, and three cases of *MLL*-associated SL were diagnosed 15 months from the start of induction. One case (patient #1) followed chemotherapy alone, as described.³¹ Two cases (patients #2 and #3) followed local RT and 3F8 immunotherapy. Three cases (patients #4–6) followed local RT, 3F8 immunotherapy, and ^{131}I -3F8-targeted RT, with one presenting 4 weeks after the infusion of ^{131}I -3F8, as described,³² and two detected 16 months and 43 months, respectively, after the infusion of ^{131}I -3F8. Two patients (#2 and #3) never received G-CSF. Two patients (#3 and #6) became long-term leukemia-free survivors, though one eventually died of NB.

In the 6-cycle group, one patient (#7 in Table III) developed SL/MDS. The child presented with circulating blasts and thrombocytopenia 6 months post-MAT. She is 14+ months post-allograft, in complete remission, off all immunosuppressive medications.

In the 5-cycle group, one patient (#8 in Table III) developed SL/MDS. Without surveillance chromosomal studies, the case would have passed undetected for at least 36 months because peripheral blood findings, BM morphology, and physical examination remained unremarkable. The patient's *MLL* translocation was not evident pre-MAT or 2 months post-MAT; it was first detected 10 months post-MAT, 2 months after completion of maintenance oral etoposide, and in the midst of a second cycle of 13-*cis*-retinoic acid. Over the next 37 months, the same *MLL* translocation was present in up to 100% of BM cells (and in up to 98% of circulating leukocytes) but BM had a low blast count and no myelodysplasia, and monthly peripheral blood had normal leukocyte, differential, and platelet (163,000–395,000/ μl) counts, and normal mean corpuscular volume. At 37 months, BM contained 13% blasts, newly-present dysplastic features, and a new subclone with monosomy 16 and additional 18q. Anti-leukemia therapy was initiated. As of December, 2008, he is 23+ months post-allograft, in complete remission, off all medications.

DISCUSSION

Reducing the number of dose-intensive induction cycles in patients with high-risk NB significantly decreases the risk of SL/MDS ($p=0.048$), yielding a 5-year cumulative incidence of ~1.5–2.3% which approximates the low range (0.4%-to-2.2%) reported for moderate-dose combination chemotherapy regimens used against pediatric solid tumors.^{10–18} This finding may help improve the event-free survival of patients with high-risk NB.

Several points about the current study merit emphasis: First, very few other studies on SL/MDS match the chemotherapy dose-intensity in the current report, which, moreover, includes 184 patients as compared to 73 patients in those other studies.^{7–10} Second, the MSKCC patients had BM studied prospectively for SL/MDS, not only when blood or BM findings raised suspicions – hence the experience might provide a more accurate measure of the risk than other studies. In fact, two cases were detected incidentally, including one (patient #8) that would have passed undiagnosed for 37 months in the absence of surveillance BM chromosomal studies at 17 months (see below). Third, the findings have far-reaching relevance and import: the recent COG national study (A3973) used 6 cycles of this induction, and induction in the successor study (ANBL0532) also uses 6 cycles (4 as in the A3973 study,⁴ 2 with a lower but still considerable dosage of cyclophosphamide [2000 mg/m²/cycle] and topotecan). Fourth, secondary findings are also of interest, due to a paucity of published data, and relate to possible leukemogenicity of treatments commonly used in patients with NB or other solid tumors, including myeloablative therapy, targeted RT, oral etoposide (4 cycles), and G-CSF (used with upfront dose-intensive chemotherapy). None of these treatments were significantly associated with an increased risk of SL/MDS.

Because so few reports on this kind of dose-intensive induction chemotherapy have been published, there are perforce limited data on its leukemogenicity.^{7–10} Yet the worrisome findings in prior reports of ~10% risk raise legitimate concerns about using that kind of treatment. Whether dose intensity increases the risk over and above cumulative doses has not been previously reported. Here we reduced the cumulative doses while maintaining upfront dose intensity and found that leukemogenesis becomes comparable to that reported for moderate-dose chemotherapy.

As previously reported,¹ several factors prompted a reduction from 7 to 5 cycles in induction. Thus, rapid responses were evident such that after 3–5 cycles, 91% of patients had complete remission in BM and 86% had >50% shrinkage of primary tumors; the overall complete/very good partial remission rate after 5 cycles (83%) was comparable to that after 7 cycles (77%).¹ Prolonged thrombocytopenia after cycles 6 and 7 sometimes caused delays in starting consolidative therapy,²² which might adversely affect long-term outcome. The above findings supported deleting cycles 6 and 7, and matched well with our wish to decrease toxicity, notably SL/MDS, in this young patient population.

Much of the vast literature on risk factors for SL/MDS focuses on lymphoma-leukemia, with very few large studies involving childhood solid tumors treated after 1990.^{7–10,13,15–17,33} In view of marked differences in treatments (agents, dosing, schedules), findings in patients treated for lymphoma-leukemia may be of limited relevance for young persons treated for a solid tumor. In the latter patient population, accumulating experience suggests that interactions between leukemogenic agents, as well as high dosing per course, enhance the risk of SL/MDS. Thus, coadministration of an alkylating agent and a topoisomerase-II inhibitor is more leukemogenic than using either agent alone.^{11,12,34} Further, as noted above, SL/MDS rates are very low (0.4% to 2.2% at 4 to 6 years) in other pediatric solid tumor patients treated with moderate doses of the chemotherapeutic agents used for NB.^{10–18} SL/MDS rates are also very low (0.5% at 5 years) after treatment for germ cell tumors with etoposide in cumulative dosages (2000 mg/m²) and schedules (100 mg/m²/

day, times 5 days) similar to those used in NB patients but combined with lower doses of other agents.^{13,35} Finally, patients with breast cancer have an increased risk of SL/MDS (albeit only 1% at 5 years) after intensified therapy (cyclophosphamide 2400 mg/m² and doxorubicin 60 mg/m², x4 courses).³⁶

Local RT has become standard of care in NB patients and in young persons with other solid tumors, so its leukemogenic role, if any, is difficult to define in this population. Local RT for NB is routinely approximately 2100 cGy and can encompass substantial BM space;^{5,6} much greater RT doses are used for other pediatric high-risk solid tumors and usually less BM space is affected. One might expect weak-to-absent leukemogenicity if local RT completely ablates BM within the RT field. One of our patients (#1 in Table III) developed SL without prior RT.

Among the 40 patients treated with ¹³¹I-3F8-targeted RT, secondary acute lymphoblastic leukemia typical of prior exposure to topoisomerase-II inhibitors³⁷ emerged in one patient (#4 in Table III) very soon (4 weeks) after ¹³¹I-3F8 therapy, suggesting no causative role, and MDS was found in two patients (#5 and #6 in Table III) at 16 and 43 months, respectively, after ¹³¹I-3F8 therapy. More cases of SL/MDS in the ¹³¹I-3F8 cohort are possible, but survivors have passed the peak SL/MDS latency period (6–8 years) of low-dose total body irradiation.^{38,39} The data show no significant association between SL/MDS and ¹³¹I-3F8-targeted RT (p=0.343).

The sole case of SL/MDS in the 5-cycle group followed consolidation with MAT and oral etoposide (patient #8 in Table III). Those treatments may have been contributing causative factors. However, the leukemogenicity of MAT is not fully defined;⁴⁰ our patient received only four cycles of oral etoposide, which contrasts with the prolonged use of oral etoposide in heavily prior-treated NB patients who developed SL/MDS.³³ The follow-up of our NB patients treated with both is now beyond the typical risk period for *MLL*-associated SL/MDS, if not for alkylator-related SL/MDS. Of note, the patient's t(4;11) (p12;q23) rearrangement has not been described in large studies of 11q23,^{20,37,41–44} and his clinical course was unusual in that 11q23 translocations are linked to early onset of overt SL whereas this patient had no hematologic, BM, or clinical signs of SL/MDS for 37 months after the incidental discovery of the translocation. The hope was that the abnormality might spontaneously resolve, as described with other SL/MDS-associated chromosomal aberrations.^{45,48} This patient's course suggests that cases of SL/MDS likely pass undetected in the absence of surveillance.

The routine use of G-CSF in oncology since the mid-1990s prevents definitive conclusions about its possible leukemogenicity with chemotherapy, but caution is warranted as recent complex analyses involving breast cancer patients and children with acute lymphoblastic leukemia point to an enhancing effect.^{36,49,50} SL/MDS developed in two of the 28 patients treated with 7 cycles but no G-CSF, and the risk of SL/MDS was low among the 121 patients treated with 5 or 6 cycles plus G-CSF. This experience suggests that G-CSF has little or no leukemogenic effect when used with short-term, dose-intensive chemotherapy and local RT.

In conclusion, using fewer than seven cycles of dose-intensive induction chemotherapy has eventuated in a low incidence of SL/MDS in our large NB population. This finding is reassuring in view of studies of serially collected BM specimens which reveal that *MLL* translocations can occur soon after initial exposure to topoisomerase II inhibitors (i.e., after only 2–3 induction doses).^{31,49}

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Figure 1. Cumulative incidence of secondary leukemia/myelodysplastic syndrome (SL/MDS) (black curves) and neuroblastoma progression (grey curves) among patients treated with 5, 6, 7, or 8 cycles of dose-intensive induction chemotherapy. Fewer cycles significantly correlated with a decreased risk of SL/MDS ($p=0.048$).

Table I

Dosing of leukemogenic agents

Agent	Cumulative dosages (mg/m ²)		
	7-cycle regimen	6-cycle regimen	5-cycle regimen
Cyclophosphamide (4200 mg/m ² /cycle [*])	16,800	16,800	12,600
Doxorubicin (75 mg/m ² /cycle)	300	300	225
Cisplatin (200 mg/m ² /cycle)	600	400	400
Etoposide (600 mg/m ² /cycle)	1800	1200	1200

* 140 mg/kg/cycle in patients up to 10 years of age

Table II

Clinical profiles

	7-cycle group*	6-cycle group	5-cycle group
Number of patients	63	45	76
Male:female (ratio)	36:27 (1.4:1)	23:22	46:30 (1.5:1)
Age (yrs) at start of therapy:			
median	3.4	3.0	3.5
range	0.3–29.1	0.5–17.2	0.3–23.2
Consolidative therapy			
Local radiotherapy	61 (97%)	45 (100%)	68 (92%)
¹³¹ I-3F8	33 (52%)	4 (9%)	3 (4%)
Myeloablative therapy	4 (6%)	36 (80%)	36 (47%)
CEM	0	27	4
TTC	1	7	32
Other	3	2	0
Oral etoposide**	0	0	22 (29%)

CEM=carboplatin (1500 mg/m²)/etoposide (2000 mg/m²)/melphalan (210 mg/m²)²⁸; TTC=thiotepa (900 mg/m²)/topotecan (10 mg/m²)/carboplatin (1500 mg/m²)²⁹;

* includes 4 patients who received 8 cycles;

** 50 mg/m²/day, x21 days, x4 cycles³⁰

Table III

Patients with secondary leukemia (SL) or myelodysplastic syndrome (MDS)

Pa- tient No./Sex	Neuroblastoma			Leukemia/Myelodysplasia		
	Induction chemotherapy	Local radiotherapy	Other therapy	Presentation (type)	Time from start of induction	Karyotype
1/M	12 yr	8 cycles	none	Monocytosis (M4)	12 mo	46, XY, del(11)(q23) ^a
2/M	3 yr	7 cycles	2100 cGy to L adrenal	Incidental ^b (M2)	15 mo	46, XY, del(9)(q13q34), del(11)(q23q25)
3/M	3 yr	7 cycles	2100 cGy to L adrenal	Thrombo- cytopenia (MDS)	27 mo	45, XY, del(5)(q11), der(7)t(7;17)(q11;q11), -17
4/M	3 yr	7 cycles	2100 cGy to T2-L1	Leukocytosis (ALL, L2)	12 mo	46, XY, t(4;11)(q21;q23)
5/F	8 yr	7 cycles	2100 cGy to skull & R adrenal	Thrombo-cytopenia (MDS)	50 mo	46, XX, dic(5;17)(q11.2;p12), der(6)t(6;7)(q13;p11.2), -13, +15, add(19)(q13), -22, +2mar
6/F	25 yr	7 cycles	2100 cGy to L adrenal	Thrombo-cytopenia (MDS)	24 mo	46, XX, del(7)(q22q32)
7/F	5 yr	6 cycles	2100 cGy to R adrenal, skull, L neck, L superior mediastinum	Thrombo- cytopenia, blasts (AML)	12 mo	46, XX, del(11)(p11;p13)
8/M	5 yr	5 cycles	2100 cGy to R superior mediastinum & L adrenal	Incidental ^c (MDS)	17 mo	46, XY, t(4;11)(p12;q23) ^d

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CEM, carboplatin-etoposide-melphalan with autologous stem-cell rescue; CRA, 13-*cis*-retinoic acid; LPD, lymphoproliferative disorder; NB, neuroblastoma; TTC, thiotepa-topotecan-carboplatin with autologous stem-cell rescue.

^a molecular studies revealed a cryptic t(11;17)(q23;p13)³¹;

^b a routine bone marrow evaluation for neuroblastoma revealed 30% blasts; peripheral blood counts were unremarkable;

^c the abnormal karyotype was discovered in a routine bone marrow evaluation for neuroblastoma; bone marrow morphology, the peripheral blood counts, and the mean corpuscular volume were unremarkable;

^d at 4 months, a clone with t(2;9)(q21;q34) appeared but persisted only 6.5 months