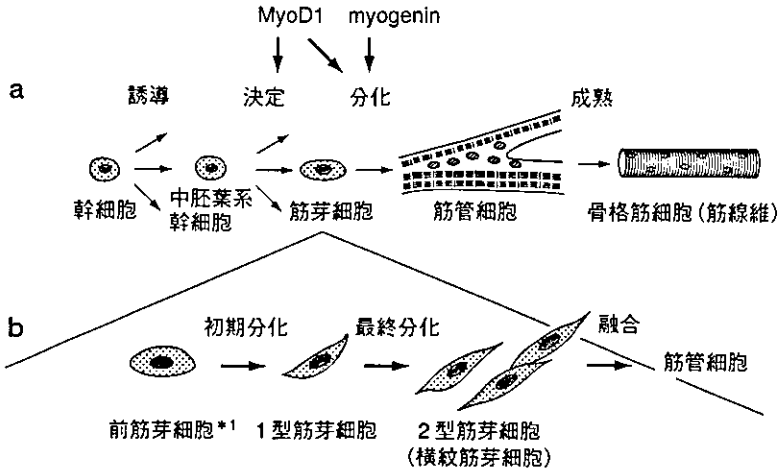

増殖能	+	+	−	−
desmin	−	+	+	+
α-sr-A*2	−	−/+	+	+
MHC*3	−	−	+	+
MHC*4	−	−	−	+

図 骨格筋の発生・分化(細井 創, 他：横紋筋肉腫の分子生物学的特性. 小児外科 26: 23-32, 1994 より一部改変)

MyoD は，多能性中胚葉系幹細胞を骨格筋細胞へと commit する「分化決定遺伝子であり，筋芽細胞を筋管細胞へ最終的に分化させる「分化誘導遺伝子」でもある。

*1: 実際には，前筋芽細胞は中胚葉系幹細胞/1型筋芽細胞と形態的には鑑別できない。*2: α-sarcomeric actin, *3: myosin heavy chain, *4: muscle creatin kinase

1）転写活性の増強および腫瘍化

胞巣型 RMS の組織内ではこれらのキメラ遺伝子の mRNA が高発現していることから，これらの遺伝子のあるレベル以上の発現によって腫瘍化が促されると考えられる[9]。*PAX3-FKHR* では PAX3 の DNA 結合ドメインが正常のまま残されているので PAX3 と同一の標的遺伝子を活性化できるが，その転写活性は通常の PAX3 のそれより 10〜100 倍高いことが示されている[10]。実際に *PAX3-FKHR* を線維芽細胞に導入すると線維芽細胞を腫瘍化させるが，PAX3 には腫瘍化能はない[11]。また，PAX3 の転写レプレッサーである KRAB を胞巣型 RMS の細胞株に導入すると増殖能が抑制される[12]。

2）キメラ遺伝子の核内偏在による活性増強

野生型の FKHR は AKT 依存性のシグナル伝達経路によってリン酸化されて核外への輸送が促されるが，キメラ遺伝子はこの制御を逃れて核内に偏在するために，DNA への結合および転写活性の増強が促されると考えられる[13]。

3）アポトーシスの抑制

一方，胞巣型 RMS の細胞株において，アンチセンスまたは KRAB を用いてキメラ遺伝子を抑制するとアポトーシス誘導により細胞死が起こることが確認されており，キメラ遺伝子によるアポトーシスの抑制もまた腫瘍化に寄与していると考えられる[14,15]。

4）筋分化の抑制

C2C12 myoblast および MyoD を導入した線維芽細胞による骨格筋細胞の分化に関する研究では，PAX3-FKHR が PAX3 よりも強く分化を抑制した[16]。また，MyoD 導入線維芽細胞ではこの抑制効果が弱いため，この分化抑制は *MyoD* の上流で起こっているものと考えられる。また NIH3T3

表 1 発生部位による臨床症候

頭頸部	
眼窩	眼球突出，眼瞼下垂，複視，眼筋麻痺
鼻咽頭	鼻閉，鼻出血，嚥下障害，腫瘤
中耳	耳漏，難聴，耳痛，顔面神経麻痺，外耳道よりポリープ状腫瘤突出
傍髄膜の頭蓋底浸潤例	頭痛，嘔吐，高血圧，髄膜刺激症状，脳神経麻痺
頸部	腫瘤，腕神経叢麻痺
泌尿生殖器	
膀胱	血尿，排尿障害，腫瘤
前立腺	腫瘤，排尿障害，便秘
膣・子宮	下腹部腫瘤，異常分泌物，外陰部からのブドウ状腫瘤突出
後腹膜	腹部腫瘤，便秘，腹水，腹痛
肛門・会陰部	ポリープ状の腫瘤
胆道	黄疸，肝腫大

細胞にレトロウイルスベクターで PAX3 あるいは PAX3-FKHR を導入し，マウス cDNA マイクロアレイで発現遺伝子の差異をスクリーニングしたところ，同レベルの発現でも PAX3-FKHR のみが MyoD と myogenin を含む多数の骨格筋分化制御因子の発現を誘導できた[17]。

5）細胞周期のブレーキ異常

最近，PAX3-FKHR が Cyclin-dependent kinase (CDK) inhibitors の一つである p27Kip1 蛋白の degradation を促進することによって細胞周期のブレーキ異常を引き起こしている可能性が報告されている[18]。

[胎児型 RMS における遺伝子異常]

胎児型 RMS においては，はっきりとした原因遺伝子は特定されていない。多くの例で染色体の 11p15.5 にアレル欠失（または loss of heterozygosity: LOH）が認められる[19]。この領域は，Beckwith-Wiedemann 症候群，Wilms 腫瘍，肝芽腫などとも関わりが深く，胎児性組織の異常増殖に関連していると考えられる。胎児型 RMS の細胞株である RD 細胞へ 11 番染色体の一部を導入する研究を行ったところ，11p15 領域の導入によって明らかな増殖抑制が認められた[20]。この部位は胎児型 RMS におけるアレル欠失の部位を含むため，この部位にがん抑制遺伝子が存在し，胎児型 RMS では同部の LOH によって腫瘍化が促されると考えられる。さらにこの LOH は，genomic imprinting の機序により，常に母親側のアレルが欠失し，父親側の不活性なアレルが残ることによって生じることもわかっている。近年，comparative genomic hybridization (CGH) や fluorescence in situ hybridization (FISH) を用いた解析により，胎児型 RMS の新たな遺伝子異常が発見されつつある[21]。

3. 臨床症候

RMS は本来骨格筋の存在しない部位を含め，身体の至る所より発生する。腫瘍が大きくなると，その発生部位に特異的なさまざまな症候が現れる。IRS-III のデータに基づく部位別の頻度としては，四肢 19%，傍髄膜 15%，泌尿生殖器 12%，眼窩 10%，傍髄膜・眼窩以外の頭頸部 10%となっている[22]。また，25%の患者は初診時に遠隔転移を有し，その半分は 1 臓器の転移である。転移部位としては肺（40～50%），骨髄（20～30%），骨（10%），リンパ節（20%）があげられる[22]。脳転移や肝転移は初発時にはまれであるが，治療抵抗性の再発患者ではときに認める。部位別の臨床症候を**表 1** に示す。

四肢原発の場合は 50～75%が胞巣型 RMS であり，血流も豊富であることから，遠隔転移の検索は必須である。さらに筋膜に沿った進展，領域リンパ節への進展にも注意が必要である。四肢以外にも泌尿生殖器，会陰部原発の腫瘍では領域リンパ節転移が多く，初診時のステージ決定および局所コントロールの範囲に注意が必要である。

4. 臨床検査

はっきりとした外傷既往がなく，皮膚変色を伴わない腫瘤をみた場合，身体のどの部位にあっても RMS は鑑別の対象となる。腫瘍マーカーとして確実なものはないが，転移性の胞巣型 RMS で

クレアチンホスホキナーゼ(CK)のMB分画が高値となる報告がある[23]。確定診断のためには組織診断が必須である。針生検では確定診断に十分な腫瘍組織が採取できないことがあるため，開放生検が原則である。上述のように病理組織では胎児型と胞巣型の2つの種類に分類され，それぞれに分子生物学的な発症機序が異なる。以下にそれぞれの形態学的特徴を述べる。

[胎児型(embryonal type)]

腫瘍細胞は小円形または紡錘形で核の多型性は少ない。細胞質は乏しく好酸性顆粒を有し，横紋のある横紋筋芽細胞がみられることがあり，診断の手掛かりとなる。一亜型としてブドウ肉腫型(botryoid type)とよばれるものがあり，肉眼的にブドウの房状の外観を示すため，このようによばれる。ブドウ肉腫型は，鼻咽頭，外耳道，泌尿生殖器，消化管に発生し，乳幼児に多い。

[胞巣型(alveolar type)]

腫瘍が組織構築上，胞巣状に増殖する。腫瘍細胞自体は未分化な小円形細胞で，隔壁に吊るした柿状に付着しているのが特徴である。胎児型と混在し，混合型と診断されることもある。四肢に比較的多く発生するのが特徴であるが，そのほか体幹，頭頸部にもみられる。周囲の組織に浸潤性に拡大し，遠隔転移しやすく，予後不良である。

組織特有な細胞骨格蛋白や細胞内蛋白は，免疫組織学的染色によって検出され，腫瘍細胞起源の同定または分化のマーカーとなりうる。筋細胞に特有な細胞骨格蛋白(desmin，αとγ-muscle actin)，骨格筋と心筋に特有な細胞骨格蛋白(α-sarcomeric actin)，骨格筋に特有な細胞内蛋白myoglobinなどが，骨格筋由来の悪性腫瘍であるRMSの鑑別に有用である。近年，MyoD1蛋白に対する抗体が開発され，未分化RMS細胞に対しても高い陽性率と特異性を示すため，RMSの組織診断のゴールド・マーカーとして用いられつつある[24]。

また，胞巣型RMSの病因である染色体転座に基づく*PAX3-FKHR*および*PAX7-FKHR*キメラ遺伝子は，RT-PCR法で検出できるため，胞巣型RMSの診断と骨髄や採取造血幹細胞中の残存・混入腫瘍の検出が可能である[25]。

治療計画，治療効果と予後を予測するためには，組織型はもちろん，腫瘍の原発部位および病期の診断が必須である。この目的のために，血液一般，生化学，検尿，胸部X線などの一般検査に加えて，腹部エコー，CT，MRI，骨サーベイランス，骨シンチ，骨髄穿刺と髄液検査(細胞診を含む)が必要である。

5. 治療目標とその手順，および症状経過，検査所見からみた予後判定

米国Intergroup Rhabdomyosarcoma Study Group(IRS)では，上記の検査によって決定された病理組織型，術前Stage分類(表2)，および術後Group分類(表3)組み合わせてlow，intermediate，highの3つのrisk groupに分け，groupごとの治療法・研究プロトコールが設定されている[26]。このようなアプローチにより，表4に示されるような良好な治療成績が得られている[27,28]。すなわち，RMSの治療目標は長期無病生存である。この目標のために，治療の有効性を保ったまま，予後良好群には毒性の少ない安全な治療，すなわち減量した化学療法および放射線療法を適用しようというのが，この治療層別化の精神である。

治療の基本は外科手術と化学療法，放射線療法の組み合わせによる集学的治療である。原発部位や腫瘍サイズ，領域リンパ節転移の有無，遠隔転移の有無(以上stage)，初回手術による原発腫瘍摘除状態，領域リンパ節転移郭清の有無(group)，組織型(胎児型か胞巣型か)などによって予後が異なるため，異なる化学療法，放射線照射量が設定されている。

[外科治療]

1) 一期的全摘除および治療前再摘除

組織学的全摘除(Group I)は，切除断端に顕微鏡的腫瘍遺残のある肉眼的全摘除(Group II)，肉眼的腫瘍遺残のある亜全摘除または部分摘除(Group III)に比し明らかに予後が良好であるので，可能な限り腫瘍を一期的に全摘除することが治療の原則である。万一，良性腫瘍を考慮して針生検あるいは生検目的の不完全切除が行われ，Group IIあるいはGroup IIIとなった時は化学療法や放射線照射前の治療前再摘除(pretreat-

表 2　横紋筋肉腫の術前 Stage 分類(IRS pre-treatment TNM staging classification)

Stage	原発部位(Sites)	T	Size	N	M
1	眼窩 頭頸部(傍髄膜を除く) 泌尿生殖器(膀胱，前立腺を除く) 胆道	T1 or T2	a or b	N0 or N1 or Nx	M0
2	膀胱・前立腺 四肢 傍髄膜 他(体幹，後腹膜，会陰・肛門周囲，胸腔内，消化管，胆道を除く肝臓)	T1 or T2	a	N0 or Nx	M0
3	膀胱・前立腺	T1 or T2	a	N1	M0
	四肢 傍髄膜 他		b	N1 or N0 or Nx	M0
4	すべて	T1 or T2	a or b	N0 or Nx	M1

1．原発腫瘍(T)　T1：原発部位に限局
T2：原発部位を越えて進展または周囲組織に癒着
2．大きさ(Size)　a：最大径で 5 cm 以下
b：最大径で 5 cm を越える
3．領域リンパ節(N)　N0：リンパ節転移なし
N1：領域リンパ節に転移あり(画像または理学所見上)
Nx：転移の有無は不明(とくに領域リンパ節転移の評価困難な部位)
4．遠隔転移　M0：なし
M1：あり

表 3　横紋筋肉腫の術後 Group 分類(IRS clinical grouping classification) (post-surgical)

Clinical Group	
I	組織学的に全摘除された限局性腫瘍 a．原発臓器または筋に限局 b．原発臓器または筋を越えて(筋膜を越えて)周囲に浸潤 ただし，いずれの場合も領域リンパ節に転移は認めない(頭頸部を除いてサンプリングまたは郭清により組織学的確認を必要とする)
II	肉眼的に全摘除された領域内進展腫瘍 a．切除断端に顕微鏡的腫瘍遺残あり，ただし領域リンパ節に転移を認めない。 b．領域リンパ節に転移を認めるが完全摘除を行った，すなわちもっとも遠位の郭清リンパ節に転移を認めない。 c．領域リンパ節に転移を認め，しかも切除断端に顕微鏡的腫瘍遺残を認めるかもっとも遠位の郭清リンパ節に転移を認める。
III	肉眼的な腫瘍遺残 a．生検のみ施行 b．亜全摘除または 50%以上の部分摘除を施行
IV	a．遠隔転移(肺，肝，骨，骨髄，脳，遠隔筋組織，遠隔リンパ節など)を認める。 b．脳脊髄液，胸水，腹水中に腫瘍細胞が存在 c．胸膜播種，腹膜(大網)播種を伴う。

注) 初回の術中所見および病理所見(化学療法，放射線療法未施行)により分類され，以後の二期手術の結果には影響されない。

ment re-excision: PRE)が推奨されている。

2）二期的待期手術(second-look operation)

ただし，治療開始の時点から，生命優先の名のもと組織や臓器の機能障害，外観の損傷など容認しがたい障害をつくり出すことは避けるべきで，group II あるいは III として 12 週計 4 クールの化学療法後の二期的待期手術(second-look operation)を目指すべきである。

[化学療法]

Low risk 群の一部で Vincristine (V)+Actino

表 4 IRS-V risk group 分類と生存率および治療法

	Low Risk Group									
	subgroup A(LA)				subgroup B(LB)					
組織型	胎児	胎児	胎児	胎児	胎児	胎児	胎児	胎児	胎児	胎児
Stage	1	1	1	2	1	1	1	2	2	3
Group	I	IIA	III	I	II	III	III	II	I，II	I，II
原発部位	良好	良好	眼窩	不良	良好	眼窩	眼窩以外	不良	不良	不良
大きさ				5 cm 以下				5 cm 以下	5 cm 以下	5 cm 以上
領域リンパ節転移		なし	なし	なし	あり	あり			あり	
年齢										
5 年無病生存率(%)	89	87	78	82	100		90	100		82
5 年生存率(%)	96	96	96	96	100		100	100		90
治療	VA 療法				VAC 療法					

	Intermediate			High	
	Int. A	Int. B	Int. C	High A	High B
組織型	胎児	胎児	胞巣	胎児	胞巣
Stage		4		4	4
Group	III	IV	I - III	IV	IV
原発部位	不良				
大きさ					
領域リンパ節転移					
年齢	10 歳未満			10 歳以上	
5 年無病生存率(%)	76	55	55		
5 年生存率(%)	83	59		30 以下	30 以下
治療	VAC と VTC 比較検討			VAC と CPT 比較検討	

*良好部位：眼窩，頭頸部(表面)，傍精巣，膣，子宮，胆道
**不良部位：傍髄膜頭頸部，膀胱，前立腺，四肢，その他

mycin D(A)療法(全治療期間 48 週)が選択されるが，標準化学療法は V+A+Cyclophosphamide(VAC)療法(全治療期間 42 週)である。IRS-V の Intermediate リスク群では，VAC 療法による標準治療群と V+C+Topotecan を併用した VTC 療法を含む治療アームのランダム化比較試験が行われている。また，依然治療成績の不良な High risk 群では，Irinotecan(CPT)の Phase II window 試験を導入し，CPT が有効である症例には VAC 療法と CPT 療法を交代で施行するレジメンとしている。

[放射線治療]

1) Low-risk 群の Group I および II

Group I 症例には照射は行わず，Group II (組織学的残存腫瘍)症例には化学療法開始後第 3 週で，領域リンパ節転移のなかった例には 36 Gy，あった例には 41.4 Gy を照射する。

2) Low risk 群の Group III および Intermediate-risk 群

第 12 週の二期的待期手術の腫瘍切除状況(完全切除，組織学的残存腫瘍，肉眼的残存腫瘍)により，それぞれ 36 Gy，41.4 Gy，50.4 Gy の 3 段階の照射量が規定されている。

3) High-risk 群

第 15 週の腫瘍切除時の状況で上記と同様に照射線量が規定されているが，遠隔転移部位には同週に 50.4 Gy の照射を規定している。

おわりに

分子遺伝学の進歩により，RMS の増殖と分化異常のメカニズムが明らかになりつつある。今後は，遺伝子という「点」を蛋白の機能という「線」で結ぶ作業，すなわち異常遺伝子産物である異常蛋白と他の蛋白との相互作用である「がん細胞の細胞内情報伝達路」の解明が必要となる。シグナル路の中心的標的蛋白を明らかにし，がんの病態を解明することは，副作用の少ない，がん細胞に特異的に作用するような新しいがん治療薬や治療法の開発に発展していく可能性を秘めている。

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Journal of Pediatric Hematology/Oncology, Vol. 24, No. 2, February 2002

Reprinted from JOURNAL OF PEDIATRIC HEMATOLOGY/ONCOLOGY
Vol. 24, No. 2 February 2002
Lippincott Williams & Wilkins Printed in U.S.A.

Phase I Study of Irinotecan in Pediatric Patients With Malignant Solid Tumors

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Purpose: To determine the dose-limiting toxicity, maximum tolerated dose, and potential efficacy of irinotecan in children with refractory malignant solid tumors.

Patients and Methods: In the present phase I clinical trial, 28 patients received irinotecan 50 to 200 mg/m^2 per day by intravenous 2-hour infusion over the course of 3 days, repeated once after an interval of 25 days. Fifty-one treatment courses were administered to these patients.

Results: Dose-limiting toxicities were observed at the dose of 200 mg/m^2 per day for 3 days. Diarrhea and hematopoietic toxicities were the dose-limiting factors, and the former required support with intravenous fluid administration. The occurrence of vomiting was variable. Decreases in clinical tumor marker levels were observed in the majority of patients who received two cycles of irinotecan 80 mg/m^2 per day to 200 mg/m^2 per day over the course of 3 days, and partial response was attained in four patients who received irinotecan in two cycles of 140 mg/m^2 per day to 200 mg/m^2 per day over the course of 3 days. Pharmacokinetic studies showed that the plasma concentration of irinotecan and its active metabolite SN-38 ranged from 93 to 2,820 ng/mL and 5.2 to 34.8 ng/mL, respectively, during 3-day infusions of irinotecan 200 mg/m^2 per day. The mean clearance of irinotecan was 14.54 L/h per m^2 (range 8.45–20.83 L/h per m^2).

Conclusion: The maximum tolerated dose was determined to be a dose of irinotecan between 160 mg/m^2 per day and 180 mg/m^2 per day administered over the course of 3 consecutive days on an inpatient basis, repeated once after 25 days off, and our results indicate that irinotecan is a promising anticancer agent that is worthy of phase II trials in pediatric solid tumors.

Key Words: Irinotecan—Phase I study—Advanced neuroblastoma—Pediatric solid tumors.

Camptothecin (CPT), an alkaloid with a novel ring structure, was first isolated from the Chinese tree *Camptotheca acuminata*. A water-soluble derivative of CPT, 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyl-camptothecin hydrochloride trihydrate (irinotecan, CPT-11), was subsequently synthesized (1), and it has been reported that irinotecan is active against lymphoma, gastric cancer, small cell lung cancer, non–small cell lung cancer, cervical cancer, epithelial ovarian cancer, colorectal cancer, and desmoplastic round blue cell tumor (2–10). Irinotecan and topotecan (11) inhibit DNA topoisomerase I, which is an essential nuclear enzyme that relaxes torsionally strained duplex DNA, enabling replication and transcription.

The in vivo activity of irinotecan has been determined in xenograft models of pediatric tumors, including neuroblastoma (12–18), primitive neuroectodermal tumor (12,15), rhabdomyosarcoma (13), and others. Because of this activity, two previous phase I trials of irinotecan in children were conducted in France and in the United States (19,20). The authors had started a pediatric phase I study of irinotecan in May 1996 in which irinotecan was administered by 3-day intravenous infusion to children with refractory malignant solid tumors, including advanced neuroblastoma, and we now present the results of it.

PATIENTS AND METHODS

Participating institutions and investigators (Appendix) were carefully selected from members of the Study Group of Japan for Treatment of Advanced Neuroblastoma based on their full experience in the treatment with high-dose chemotherapy of the group (21,22).

Patients aged from 3 to 19 (median 8) years at the time of diagnosis of histologically confirmed solid tumors that were deemed to be treatment failures on conventional treatment were eligible for this trial (Table 1). Other eligibility criteria included a life expectancy of at least 3 months, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1 (23,24), at least 4 weeks since and recovery from the toxic effects of previous chemotherapy, hemoglobin count >8.0 g/dL, granulocyte count >1,200/mm^3, and

Submitted for publication July 12, 2000; accepted February 19, 2001.

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Supported in part by a Grant-in-Aid for Cancer Research (9–14) from the Ministry of Health, Labor, and Welfare of the Government of Japan, and a Grant-in-Aid (1999) from the Children's Cancer Association of Japan.

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TABLE 1. *Patient characteristics*

Male/female	14/14
Age, years	
Median	8
Range	3–19
Diagnosis	
Neuroblastoma	26
Leiomyosarcoma	1
Primitive neuroectodermal tumor	1

platelet count >70,000/mm^3. Other requirements included normal liver function (bilirubin <1.5 mg/dL, aspartate transaminase and alanine transaminase less than twice the normal level), adequate renal function (serum creatinine <1.2 mg/dL). Patients with active infection, diarrhea, intestinal obstruction, pleural fluid or ascites, pneumonitis or pulmonary fibrosis, uncontrollable diabetes, and allergic reaction were excluded from this study. It was originally planned to evaluate three patients at each dosage level and to increase the dose to the next level when toxicities described later in this article were observed in none or only one of the three patients. However, when the first two patients showed no toxicities, the dose was increased to the next level. There was no intrapatient increase of dosage. Written informed consent for participation was obtained from all patients or their guardians, and the study protocol approved by the institutional review boards of all participating institutions.

Patients at each dosage level were evaluated for nonhematopoietic toxicity with the ECOG common toxicity criteria (25) and for hematopoietic toxicity with a table of hematopoietic toxicities modified by the current study group from the ECOG criteria (Table 2). The idea of this modification derived from the fact that all of the patients in the current study were recipients of prolonged high-dose chemotherapy and their granulocyte and platelet counts were only slightly more than 1,200/mm^3 and 70,000/mm^3, respectively. The maximum tolerated dose (MTD) was defined as the dose level less than that which caused three or more (50% or more) of six patients to experience grade 4 hematopoietic or grade 3 nonhematopoietic toxicity, excluding vomiting, and it should also be the dose that patients tolerated without significant difficulty and without any intensive support. The treatment course was repeated once after an interval of 25 days in the absence of dose-limiting toxicity. This schedule of administrating irinotecan over the course of 3 consecutive days for two courses with 25 days off was chosen for two reasons based on our basic and clinical studies: first, it was presumed that repeated administration over the course of 3 days might be more effective than single bolus injection on 1 day (12,14); and second, we preferred administration of irinotecan every 4 weeks in clinical setting, as we did in previous clinical trials (21,22), and hoped the 3-day course of irinotecan could be used twice in the future protocols. Assessment of toxicities after the first of the two courses placed more importance on dose increase than assessment after the second course. Patients were removed from the study if there was evidence of progressive disease.

All treatment was conducted on an inpatient basis. Irinotecan was administered over the course of 3 consecutive days by 2-hour intravenous infusion. The starting dose was 60 mg/m^2 per day × 3 days, and the dosages were increased to 70 mg/m^2 per day × 3 days, 80 mg/m^2 per day × 3 days, 90 mg/m^2 per day × 3 days, 100 mg/m^2 per day × 3 days, 120 mg/m^2 per day × 3 days, 140 mg/m^2 per day × 3 days, 160 mg/m^2 per day × 3 days, 180 mg/m^2 per day × 3 days, and 200 mg/m^2 per day × 3 days.

Before enrollment in this phase I study, participating investigators were requested to obtain an informed consent from the patient or the guardian, to complete a form with pertinent information about each patient, and to send it to the coordinating office at the University of Tsukuba, Tsukuba, Japan by facsimile for approval for entry. Irinotecan was purchased from Yakult Honsha, (Yakult Honsha, Tokyo, Japan) and from Daiichi Pharmaceutical (Daiichi Pharmaceutical, Tokyo, Japan) and distributed to individual participating investigators from the Gunma Children's Medical Center, Gunma, Japan, on entry approval. Irinotecan was predissolved in a solvent at a concentration of 40 mg/2 mL and was stored in vials. Irinotecan in vials is stable at room temperature, and it was further diluted at the time of administration to patients. No particular selection of

TABLE 2. *Nonhematopoietic toxicities of the ECOG common toxicity criteria (25) and hematopoietic toxicities modified from the ECOG common toxicity criteria for the present study*

Grade	0	1	2	3	4
Serum aspartate transaminase	<1.5 × nL	1.5–2 × nL	2.1–5 × nL	>5 × nL	—
Alkaline phosphatase	<1.5 × nL	1.5–2 × nL	2.1–5 × nL	>5 × nL	—
Bilirubin	<1.5 × nL	1.5–2 × nL	2.1–5 × nL	>5 × nL	—
Nausea and vomiting	None	Nausea	Controllable	Intractable	—
Diarrhea	None	2–3 times increase in stool	4–6 times increase in stool	7–9 times increase in stool	>10 times increase in stool, bloody stool
White blood cell count (×10^3/mm^3)	>4.0	3.9–2.5	2.4–1.5	1.4–0.5	<0.4
Granulocyte count (×10^3/mm^3)	>2.0	1.9–1.3	1.2–0.8	0.7–0.3	<0.2
Platelet count (×10^3/mm^3)	>100	99–70	69–40	39–20	<19

nL, upper border of the normal ranges at individual institutions.

patients was made, and patients were enrolled according to the order of the time of entry.

Before the study, a complete history of each patient was performed and each underwent a physical examination, including documentation of measurable lesions. Laboratory studies included complete blood cell counts, serum bilirubin, aspartate transaminase, alanine transaminase, creatinine, and others. To assess toxicities, the time of onset and degree of nausea, vomiting, diarrhea, anorexia, alopecia, anemia, leukopenia, granulocytopenia, thrombocytopenia, and abnormalities of liver and kidney function were recorded.

Blood samples for pharmacokinetic studies were collected in heparinized tubes immediately before the start of infusion, immediately after infusion (approximately 2 hours from the start of infusion), at 4, 8, and 22 hours after infusion on days 1 and 3 of each 3-day course. All samples were immediately centrifuged, and the sera were stored at −80°C until assay. Specimen processing, extraction, and high-performance liquid chromatography for quantification of total CPT-11 and SN-38 were performed at the Bio Medical Laboratories, Tokyo, Japan, using a modification of the previously reported method (18,26). The diluted samples were applied to a C18 cassette of an advanced automated sample processor (Analytichem International, Harbor City, CA, U.S.A.). A high-performance liquid chromatography column (LC-4A; Shimadzu Seisakusho, Kyoto, Japan) was linked to the advanced automated sample processor and a C18 reverse-phase column (LiChrosorb RP-18; 25 × 0.4 cm; Merck, Darmstadt, Germany) with an RP-18 precolumn was used for chromatography. The mobile phase consisted of acetonitrile/ethanol per 0.8% ammonium carbonate (2:1:1 volume-to-volume ratio) and acetonitrite/water (1:2 volume-to-volume ratio) for CPT-11 and SN-38, respectively. The flow rates and column temperatures were 1.0 mL/min and 50°C for CPT-11 and 1.5 mL/min and 60°C for SN-38. A fluorospectromonitor (Model RF-535; Shimadzu Seisakusho) was set at an excitation wavelength of 373 nm for CPT-11 and at 540 nm for SN-38. The peak heights were integrated by a data processor (Chromatopac C-RIBS; Shimadzu Seisakusho).

Pharmacokinetic parameters were calculated by the noncompartmental analysis with WinNonlin (Pharsight Corp., Mountain View, CA, U.S.A.). Response to irinotecan was classified as complete response, partial response, stable disease, and progressive disease, as originally categorized in the World Health Organization handbook (27). For example, partial response was defined as at least 30% decrease in the longest diameter or at least 50% decrease in the largest area by two-dimension measurements at 4 weeks.

RESULTS

A total of 51 3-day courses were administered to the 28 patients enrolled in this study, and all patients were evaluable for response and toxicity. Diarrhea and myelosuppressive toxicity were dose-limiting, and myelosuppression involved all marrow hematopoietic lineages (Table 3). The occurrence of vomiting was variable and was not dose-limiting (Table 3). Diarrhea and myelosuppression were more intense after the second course than after the first course in six of 23 patients who received two courses of irinotecan, less intense after the second course in one of the 23 patients, and were approximately of the same intensity in the remaining 16 patients, irrespective of the course.

Abnormalities in the liver function test results were observed in four of the 28 patients. They were observed in one patient who received irinotecan 60 mg/m^2 per day or 200 mg/m^2 per day over the course of 3 days and in two patients who received irinotecan 180 mg/m^2 per day over the course of 3 days. Grade 2 abnormality of serum aspartate transaminase (Table 2) was observed in three patients: grade 1 abnormality of serum alkaline phosphatase in one, but serum bilirubin was normal in all of them. As a matter of course, abnormalities in the liver function test results were of grade 1 or 2, and not dose-limiting at all in the current study. Vomiting of grade 2 and 3 was observed in 17 of the 28 patients but was not so severe as that in the case

TABLE 3. *Nadir of leukocytes, granulocytes, and platelets, and number of grade 4 hematopoietic and grade 3 nonhematopoietic toxicities by irinotecan dose*

Dosage (mg/m^2 × 3 d)	No. of patients	No. of courses	Leukocyte nadir (/mm^3)	Granulocyte nadir (/mm^3)	Platelet nadir (×10^3/mm^3)	Grade 4 hematopoietic toxicity/course	Grade 3 nonhematopoietic toxicity (diarrhea)/course
60	3	6	1,300–3,400	616–1,107	66–155	0/6	0/6
70	3	5	1,000–2,600	492–792	38–247	0/5	0/5
80	5	10	720–3,500	86–1,278	20–153	2*/10	0/10
90	2	4	200–4,100	1,040–1,394	7–115	2*/4	0/4
100	2	4	1,100–1,700	348–663	37–128	0/4	0/4
120	2	3	1,400–2,800	574–1,300	27–96	0/3	0/3
140	2	4	1,300–2,300	266–884	41–90	0/4	2*/4
160	2	4	500–1,000	35–296	16–31	2†/4	1‡/4
180	4	5	500–1,400	110–539	27–67	2/5	1/5
200	3	6	200–1,200	0–144	5–112	4/6	2/6

*One of the two grade 4 or 3 toxicities was seen after the second course.
†Both were seen after the second course.
‡This was seen after the second course.

of administration of *cis*-platinum or high-dose cyclophosphamide. Other complications were of minor degree in all. There were no deaths within 30 days after the last course of irinotecan treatment.

The dose-limiting toxicity was found in more than 50% of the patients who received the dose of irinotecan of 200 mg/m^2 per day administered over the course of 3 consecutive days, repeated once after 25 days off. All of the first three patients already experienced grade 4 hematopoietic or grade 3 nonhematopoietic toxicity, excluding vomiting, and it was already certain that 50% or more of the patients at this dose level experienced such toxicities, whether there were three patients or six patients. After finding that the dose of 180 mg/m^2 per day is the dose level immediately below the level at which more than 50% experienced dose-limiting toxicity, two more patients were enrolled at this dose to find the MTD of irinotecan in children. As a result, we could observe toxicities in two patients with the dose of 160 mg/m^2 per day for 3 days and in four patients with the dose of 180 mg/m^2 per day for 3 days. Among these six patients (nine courses), grade 4 hematopoietic toxicities were found in four of nine courses, and grade 3 nonhematopoietic toxicities (diarrhea) in two of the nine courses. Nevertheless, the degree of the hematopoietic toxicities was relatively minor; it was found that the nadir of granulocyte count ranged from 35/mm^3 to 539/mm^3 (median 261/mm^3) and that of platelet count ranged from 16 × 10^3/mm^3 to 67 × 10^3/mm^3 (median 31 × 10^3/mm^3) in these nine courses of the treatment. The authors found that all hematopoietic and nonhematopoietic toxicities observed in these six patients are tolerable in so far as the children are treated on an inpatient basis, and the MTD for hospitalized children was defined to be a dose of irinotecan between 160 mg/m^2 per day and 180 mg/m^2 per day administered for 3 consecutive days. Also of note is that the nadir of granulocyte count ranged from 0/mm^3 to 144/mm^3 (median 88/mm^3) and that of platelet count ranged from 5 × 10^3/mm^3 to 112 × 10^3/mm^3 (median 15 × 10^3/mm^3) in six courses of the three patients who received irinotecan 200 mg/m^2 per day for 3 days.

Pharmacokinetic studies were attempted in 12 patients, but only successfully conducted in seven patients who received different doses of irinotecan (Table 4), and showed that the plasma concentration of irinotecan and its active metabolite SN-38 ranged from 93 to 2,820 ng/mL and 5.2 to 34.8 ng/mL, respectively, during infusions of 200 mg/m^2 per day of irinotecan over the course of 3 days (Fig. 1). Plasma CPT-11 and SN-38 levels of the patients were higher on day 3 than they were on day 1, being in the dose range from 80 mg/m^2 per day over the course of 3 days to 200 mg/m^2 per day over the course of 3 days (Fig. 1). The mean clearance of irinotecan was 14.54 L/h per m^2 (range 8.45–20.83 L/h per m^2), and the mean half-life was 6.95 hours (range 4.29–11.16 hours). Higher area-under-the-curve values of irinotecan and SN-38 were observed when the dose of irinotecan increased (Table 4).

Decreases in levels of tumor markers such as urinary vanillylmandelic acid and homovanillic acid were observed in the majority of the patients who received more than irinotecan 80 mg/m^2 per day over the course of 3 days (Table 5). However, it appeared that the response depended not only on the dose but also on the aggressiveness of the refractory disease, and there was a general tendency for more patients to show responses with higher doses (Table 5). A partial response consisting of a significant decrease in tumor size (27) and in tumor marker levels was observed in four children who received irinotecan 140 mg/m^2 per day over the course of 3 days, 160 mg/m^2 per day over the course of 3 days, and 200 mg/m^2 per day over the course of 3 days, respectively. Three of these four patients were children with advanced neuroblastoma, and the remaining one patient had leiomyosarcoma (Table 5).

DISCUSSION

In this phase I study of irinotecan in children, entry was originally open to children with all kinds of malignant solid tumors, but 26 (92.9%) of the 28 children were patients with advanced neuroblastoma, presumably because this phase I study was conducted among member institutions of the Study Group of Japan for Treatment of Advanced Neuroblastoma (Appendix). Patients generally tolerated the infusion without difficulty on an inpatient basis. Myelosuppression and diarrhea were found to be dose-limiting factors, and the dose-limiting toxicity dose was concluded to be 200 mg/m^2 per day over the course of 3 consecutive days.

TABLE 4. *Pharmakokinetic studies of irinotecan in seven patients*

Patients	1	2	3	4	5	6-1	6-3	7-1	7-3
Dose of irinotecan	A	A	A	A	B	C	C	C	C
CPT-11									
Half-life (h)	11.16	4.90	7.11	7.83	8.33	4.29	5.16	6.63	7.01
AUC (μg × h/mL)	3.83	7.10	4.25	3.36	6.35	11.97	17.89	10.42	13.56
Clearance (L/h × m^2)	13.05	8.45	14.13	20.83	12.59	16.70	11.18	19.20	14.75
Vdss (L/m^2)	154.2	56.5	141.7	177.5	87.9	56.5	51.1	135.2	99.1
SN-38									
Half life (h)	9.25	7.40	8.29	8.42	5.59	13.85	17.74	16.43	10.39
AUC (ng × h/mL)	49.5	163.5	108.9	178.2	175.3	319.0	504	183.6	325.5

6-1, day 1 of patient 6; 6-3, day 3 of patient 6; A, 60–80 mg/m^2 per day; B, 90 mg/m^2 per day; C, 180–200 mg/m^2 per day; AUC, area under curve; Vdss, volume distribution.

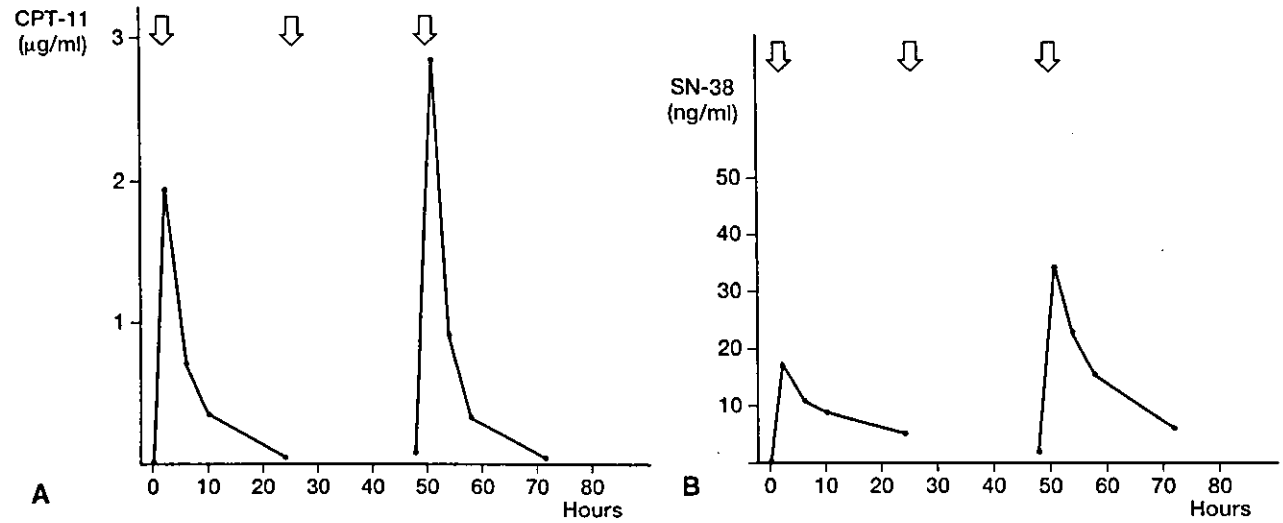

FIG. 1. Plasma concentration profiles of CPT-11 **(A)** and SN-38 **(B)** are shown. The patient was administered 200 mg/m^3 irinotecan for 3 consecutive days. Samples were taken during the second of the two courses. Arrow indicates time of 2-hour intravenous administration of irinotecan.

There have been other concurrent phase I trials of irinotecan for children (19,20,28), but the mode of administration differed from ours. Vassal et al. (29) reported that the MTD of irinotecan for children was 600 mg/m^2 when administered as a 120-minute intravenous infusion every 21 days. They further reported that 350 mg/m^2 is the MTD in adults undergoing the same administration schedule (19). Furman et al. (20) administered irinotecan more consecutively and found an MTD of irinotecan 20 mg/m^2 per day for 5 days, repeated once after 2 days off (total: 10 days of administration). A Japanese phase I study of irinotecan in adults reported that the MTD could be >250 mg/m^2 when administered as a single intravenous infusion, but did not report the upper limit of the tolerated dose (30). It appears unreasonable to suggest a recommended dose for further studies without confirming the actual MTD.

Mean irinotecan clearance of 14.54 L/h per m^2 (range 8.45–20.83 L/h per m^2) from our study is comparable with data reported by Vassal (29), who noted an irinotecan clearance in children (mean ± SD, 18.9 ± 9.1 L/hr per m^2) and in adults (15 L/h per m^2). Values of area under the curve of irinotecan and SN-38 were higher compared with those data reported by Furman et al. (20). In the current study, the values of area under the curve of irinotecan and SN-38 showed a general tendency to increase in accordance with dose increase of irinotecan (Table 4).

TABLE 5. *Antitumor activity of irinotecan by dose*

Dose (mg/m^2 × 3 d)	No. of patients	Number of PR	SD(TMt ↓)	SD	PD	ND
60	3		1	1		1
70	3			3		
80	5		4		1	
90	2		2			
100	2		1		1	
120	2		2			
140	2	1	1			
160	2	1	1			
180	4		2		2	
200	3	2				1

PR, partial response; SD(TMt ↓), stable disease but with transient decrease in tumor marker levels; SD, stable disease; PD, progressive disease; ND, not definitive.

The different modes of administration were designed for phase I trials, either based on the experimental results (13, 16,20,28) or with future use in clinical protocols in mind, as in the current study. Though the present authors were conducting this phase I study, Zamboni et al. (31) found that in an in vivo study, greater antitumor activity was observed after oral administration of irinotecan than after intravenous administration. One of the present authors and his coworkers (18) were able to confirm those results (31), but it was only after the present phase I study already started in May 1996. We found that a nearly eight-fold dose could be administered to mice orally and that higher plasma levels of irinotecan and SN-38 were prolonged in mice administered irinotecan orally compared with those administered it intraperitoneally (18). The 3-day course of administration was originally designed in the current study, hoping that sustained high-plasma levels of irinotecan and SN-38 could be achieved for 3 days because of a cumulative effect. Our pharmacokinetic study presented informative data for the future clinical study. It was shown that increased plasma levels of both irinotecan and SN-38 returned to the nearly normal ranges 22 hours after infusion on day 1, and these values were slightly higher on day 3 (Fig. 1). However, we consider, based on our own in vivo study (18) and on studies by others (20,31), that sustained high levels of CPT-11 and SN-38 would be desirable for tumor regression, and we wish that infusion of irinotecan for longer than 2 hours be explored in future.

Significant clinical effects of irinotecan were observed in four patients with refractory leiomyosarcoma and neuroblastomas who received 140 mg/m^2 per day, 160 mg/m^2 per day, and 200 mg/m^2 per day for 3 days, respectively, repeated once after 25 days off. Rosoff and Bayliff (10) recently reported a successful response to irinotecan in two patients aged 16 years and 18 years with desmoplastic round blue cell tumors, who received irinotecan 50 mg/m^2 per day for 5 days. They reported that the tumors became stable after five cycles and two cycles of the 5-day course, respectively (10). The results reported by others (10,20) and our present results appear to encourage the clinical use of irinotecan for patients with advanced neuroblastoma and other pediatric solid tumors.

Myelosuppression and diarrhea were generally, but not always, more intense after the second course of irinotecan administration rather than after the first course in the current study, even though the criteria for starting the second cycle was the same as that for starting the first cycle. We do not know the reason, and the pharmacokinetics study showed no cumulative effects because plasma levels of irinotecan and SN-38 returned to zero levels at the start of the second cycle (Fig. 1). As a matter of course, antitumor efficacy of two courses of irinotecan is greater than that of a single course. The recommended dose for pediatric phase II trials could be a dose between 160 mg/m^2 and 180 mg/m^2 over the course of 3 days, repeated once at an interval of 25 days.

We regret that we increased in increments of 10% to 18% as opposed to the standard 25% to 30%. Because of this, interpatient variability was likely to exceed such narrow dose increments, as it is clearly shown in Table 4 that area under the curve was sometimes greater in patients who received lower-dose CPT-11 by an increment of 10 to 20 mg/m^2 per day.

Acknowledgments: The authors are deeply grateful to Drs. K. Kawa, H. Shichino, N. Ohnuma, T. Suzuki, S. Okuzono, Y. Sasazaki, S. Noda, Y. Horigoshi, K. Sakashita, M. Inoue, M. Yasui, T. Okamura, Y. Umeda, and T. Hamazaki for their collaboration. They are particularly grateful to Drs. N. Saijo, Dr. Y. Tsunematsu, and H. Ikeda for their valuable advice on conducting the current study. Thanks are also due to Dr. T. Kurokawa, Dr. K. Terada, Dr. N. Kaneda, Dr. K. Kakihata, Mr. E. Nagai, Mr. H. Hagihara, and Mr. S. Takahashi, for their advice, and to Ms. C. Yenches for editorial assistance.

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Journal of Pediatric Hematology/Oncology, Vol. 24, No. 8, November 2002

Original Articles

Intensified Chemotherapy Increases the Survival Rates in Patients With Stage 4 Neuroblastoma With *MYCN* Amplification

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Purpose: Patients with high-risk neuroblastoma who have multiple copies of *MYCN* fare much worse than do those without *MYCN* amplification; however, it has not been clarified whether intensified chemotherapy with or without blood stem cell transplantation can alter the extremely poor prognosis of patients with amplified *MYCN*.

Methods and Results: Between 1985 and 1999, 301 patients older than age 12 months with stage 4 neuroblastoma were treated. From January 1985 to February 1991, 80 patients with stage 4 neuroblastoma with and without *MYCN* amplification uniformly received induction chemotherapy with regimen A_1 (cyclophosphamide 1,200 mg/m^2 and vincristine 1.5 mg/m^2 on day 1, tetrahydropyranyl [THP]-Adriamycin 40 mg/m^2 on day 3, and cisplatin 90 mg/m^2 on day 5). Among 22 patients with *MYCN* amplification, nine (40.9%) achieved a complete remission and seven (31.8%) underwent stem cell transplantation. Of 58 patients without *MYCN* amplification, 43 (74.1%) achieved a complete remission and 14 (24.1%) underwent stem cell transplantation. The 5-year relapse-free survival rates were 23.2% for stage 4 patients with *MYCN* amplification and 33.3% for those without *MYCN* amplification ($P = 0.029$); the 5-year overall survival rates were 32.8% for stage 4 patients with *MYCN* amplification and 42.8% for those without *MYCN* amplification ($P > 0.05$). From March 1991 to June 1998, patients with stage 4 neuroblastoma who had 10 or more copies of *MYCN* were treated with regimen A_3 (cyclophosphamide 1,200 mg/m^2 per day on days 1 and 2, THP-Adriamycin 40 mg/m^2 on day 3, etoposide 100 mg/m^2 per day on days 1 to 5, and cisplatin 25 mg/m^2 per day on days 1 to 5); those with fewer than 10 copies of *MYCN* received regimen new A_1 (cyclophosphamide 1,200 mg/m^2 on day 1, THP-Adriamycin 40 mg/m^2 on day 3, etoposide 100 mg/m^2 per day on days 1 to 5, and cisplatin 90 mg/m^2 on day 5), which is similar in intensity to regimen A_1. Among 88 patients with *MYCN* amplification, 63 (71.6%) achieved a complete remission and 63 (71.68%) underwent stem cell transplantation. Of 133 patients without *MYCN* amplification, 93 (69.9%) achieved a complete remission and 71 (53.4%) underwent stem cell transplantation. The 5-year relapse-free survival rates were 36.0% for stage 4 patients with *MYCN* amplification and 32.2% for those without *MYCN* amplification ($P > 0.05$), the 5-year overall survival rates were 34.0% for stage 4 patients with *MYCN* amplification and 38.9% for those without *MYCN* amplification ($P > 0.05$). The difference in relapse-free survival rates was significantly different ($P = 0.003$) between patients with *MYCN*-amplified tumor treated before (regimen A_1) versus after 1991 (regimen A_3).

Conclusions: With the use of the more intensive induction regimen A_3 plus blood stem cell transplantation for *MYCN*-amplified patients, survival curves for those with or without *MYCN* amplification now appear similar. Higher doses of chemotherapy may ameliorate the effect of *MYCN* amplification in patients with high-risk neuroblastoma.

Key Words: Neuroblastoma—*MYCN* amplification—High-dose chemotherapy.

In 1985, Seeger et al. (1) reported that multiple copies of the *MYCN* oncogene were associated with rapid progression of neuroblastomas. They reported that patients with stage 4 tumors with *MYCN* amplification had the most rapid disease progression and that the 9-month progression-free survival rate was 0% in patients whose tumors contained 10 or more copies of the *MYCN* oncogene. As a result, it was conjectured that amplification of the *MYCN* oncogene might play a key role in determining the aggressiveness of neuro-

Submitted for publication January 26, 2001; accepted June 25, 2001.

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Supported in part by Grants-in-Aid for Cancer Research (9–14 and 13–20) from the Ministry of Health, Labour, and Welfare of the Government of Japan.

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blastomas. Since that first report, attempts have been made to determine the mechanism of rapid progression of tumors with *MYCN* amplification. It was found that MYCN-MAX heterodimers form when there is an excess of the protein encoded by *MYCN*, and that this heterodimeric protein complex activates transcription of several genes likely involved in cellular proliferation (2,3).

In 1990, we reported clinical results comparable with those of Seeger et al. (1) but with improved survival rates for patients with both *MYCN*-amplified and nonamplified neuroblastoma. We concluded that stage 4 neuroblastoma patients with amplified *MYCN* did not always die, but that their prognosis was still worse than that of those without *MYCN* amplification (4). Improved survival rates for patients with neuroblastoma with *MYCN* amplification were recently reported by others (5,6) and by us (7). According to Matthay et al. (6), the 3-year relapse-free survival rates after the first randomization were 27% for all patients, 34 ± 4% for those assigned to autologous bone marrow transplantation, and 22 ± 4% for those assigned to continuation chemotherapy.

In 1991, we decided to administer more intensive chemotherapy to patients with neuroblastoma who were older than age 12 months and had amplification of *MYCN* to improve the clinical results in this group and to investigate the role of *MYCN* oncogene amplification in the prognosis in the high-dose chemotherapy setting. No other protocol has stratified the treatment of patients with stage 4 neuroblastoma based on *MYCN* amplification status.

PATIENTS AND METHODS

All 301 children older than age 12 months with stage 4 neuroblastoma treated at 40 institutions in Japan (see Appendix) between January 1985 and June 1999 were enrolled and analyzed. The staging workup included bone scan, bone marrow status, catecholamine and ferritin levels, Japanese pathology classification, and MIBG scanning, if available. Clinical stage was classified or reclassified according to the International Neuroblastoma Staging System (8). The Japanese pathology classification is different from the Shimada classification (9) or the Shimada system (10), but the current study enrolled patients with histology of neuroblastoma and ganglioneuroblastoma by the Japanese classifications, which are equivalent to neuroblastoma (undifferentiated, poorly differentiated, and differentiating) and ganglioneuroblastoma (intermixed and nodular) by the Shimada system (10).

The induction chemotherapy regimens used are summarized in Table 1. From January 1985 to February 1991, all stage 4 patients uniformly received induction chemotherapy with six cycles of regimen A_1, whether *MYCN* was amplified or not (4,11). Between March 1991 and June 1999, regimens designated A_3 and new A_1 were used. Pretreatment biopsy was required for all patients, and all stage 4 patients received one cycle of regimen new A_1 while awaiting the results of Southern blot analysis of the *MYCN* oncogene. When the tumor was found to contain 10 or more copies of *MYCN*, patients received five courses of regimen A_3 until a total of six cycles was reached. In patients with less than 10 copies of *MYCN*, further courses of regimen new A_1 were administered until a total of six cycles was reached.

TABLE 1. *Chemotherapeutic regimens*

January 1985 to February 1991
Regimen A_1
Cyclophosphamide* 1,200 mg/m^2 on day 1
Vincristine 1.5 mg/m^2 on day 1
THP-Adriamycin 40 mg/m^2 on day 3
Cisplatin 90 mg/m^2 on day 5
March 1991 to June 1998
Regimen A_3
Cyclophosphamide* 1,200 mg/m^2 per day on days 1 and 2
THP-Adriamycin 40 mg/m^2 on day 3
Etoposide 100 mg/m^2 per day on days 1 to 5
Cisplatin 25 mg/m^2 per day on days 1 to 5 (continuous)
Regimen new A_1
Cyclophosphamide* 1,200 mg/m^2 on day 1
THP-Adriamycin 40 mg/m^2 on day 3
Etoposide 100 mg/m^2 per day on days 1 to 5
Cisplatin 90 mg/m^2 on day 5

*Administered as intravenous infusion over the course of 6 hours.
THP-Adriamycin = tetra-hydropyranyl Adriamycin (Nihon Kayaku, Tokyo, Japan).

Throughout the study, radical surgery was performed after the third and before the sixth cycle of chemotherapy. After completing six cycles of induction chemotherapy with these regimens, patients received continuation chemotherapy or myeloablative preconditioning regimens and then autologous bone marrow transplantation (ABMT) or peripheral blood stem cell transplantation (PBSCT), as reported previously (12). The preconditioning regimen most frequently used included melphalan 140 mg/m^2 and 90 mg/m^2 on successive days, cisplatin 90 mg/m^2, and tetrahydropyranyl Adriamycin 45 mg/m^2, with or without etoposide 200 mg/m^2 for 4 days (Table 2). The next most frequently used preconditioning regimen consisted of melphalan 180 mg/m^2 with or without etoposide 200 mg/m^2 for 4 days. Total body irradiation to a total of 10 Gy was administered at institutional discretion. Researchers were encouraged to perform ABMT/PBSCT on patients with *MYCN* amplification when in complete remission (4,12).

Amplification of the genomic DNA sequence of *MYCN* was determined (13) using the Southern blot technique at the Special Reference Laboratories, Tokyo, Japan. DNA (10 μg) was digested completely with EcoRI, electrophoresed in 0.8% agarose gel, and blotted onto a nylon filter membrane (Zeta-Probe; Bio-Rad, Hercules, CA, U.S.A.) using the standard Southern blot method. Hybridization was performed with an EcoRI fragment containing the first exon of the human *MYCN* gene. The probe was labeled with α phosphorus-32 dCTP.

TABLE 2. *Conditioning regimens for ABMT/PBSCT*

Conditioning regimen P
Melphalan 140 mg/m² on day −5, 90 mg/m² on day −4
Cisplatin 90 mg/m² on day −7
THP-Adriamycin 45 mg/m² on day −6
±Etoposide 200 mg/m² per day on days −9 to −6
±Total body irradiation 10/3 Gy on days −3, −2, −1
Conditioning regimen Q
Melphalan 180 mg/m² on day −4
±Etoposide 200 mg/m² per day on days −8 to −5
±Total body irradiation 10/3 Gy on days −3, −2, −1
Conditioning regimen R (HiMEC)
Melphalan 100 mg/m² on days −5 and −4
Etoposide 100 mg/m² per day on days −8 to −4
Carboplatin 300 mg/m² per day on days −8 to −4
±Total body irradiation 10/3 Gy on days −3, −2, −1
Conditioning regimen S*
Regimen S-1:
Ifosfamide 2.5 g/m² per day on days −8 to −4
MESNA 100% rescue on days −8 to −4
Melphalan 140 mg/m² on day −3, 70 mg/m² on day −2
Regimen S-2:
Busulfan 5 mg/m² per day on days −7 to −4
Thiotepa 400 mg/m² on days −3 and −2

THP-Adriamycin = tetra-hydropyranyl Adriamycin (Nihon Kayaku, Tokyo, Japan).
*Regimen used as tandem ABMT/PBSCT.

Data on the 301 patients were collected in March 2000, and the clinical results of patients with *MYCN*-amplified or nonamplified neuroblastoma treated before and after March 1991 were compared. The χ^2 test, Cochran–Armitage trend test, and Fisher exact method were used for statistical analyses. $P < 0.05$ was regarded as significant.

RESULTS

The median follow-up periods were 64.8 months for patients treated before March 1991 and 37.9 months for patients treated after March 1991. Twenty-two *MYCN*-amplified (mean age, 3 years 11 months) and 58 nonamplified patients (mean age, 4 years 7 months) were enrolled in the study between January 1985 and February 1991, all receiving induction chemotherapy with regimen A_1. Radical surgery was performed in 14 (63.6%) of the 22 patients with *MYCN* amplification and in 52 (89.7%) of the 58 patients without it (Table 3). These 80 patients experienced myelosuppression and other complications of induction chemotherapy, as shown in Table 4. Nine (40.9%) in the former group achieved a complete remission (14), compared with 43 (74.1%) in the latter group. In 7 (31.8%) of the 22 patients with *MYCN* amplification, ABMT/PBSCT was performed, compared with 14 (24.1%) of the 58 without it. The 5-year overall survival rates for the groups are shown in Figure 1. The overall survival rate of patients with nonamplified tumors was superior to that of those with *MYCN*-amplified tumors. However, because of two deaths from progressive disease after 120 months in the former group, the statistical difference in survival became insignificant ($P = 0.062$). The 5-year relapse-free survival rates were 23.2% for stage 4 patients with *MYCN* amplification and 33.3% for those without it (Fig. 2). The difference in the survival rate between the two groups was statistically significant ($P = 0.029$).

There were 88 *MYCN*-amplified patients (mean age, 2 years 8 months) and 133 nonamplified patients (mean age,

TABLE 3. *Stage 4 patients older than age 12 months: response rates after completion of first six cycles of chemotherapy and 5-year survival rates*

	January 1985 to February 1991	January 1985 to February 1991	March 1991 to June 1999	March 1991 to June 1999
MYCN amplification	Yes	No	Yes	No
Induction regimen	A_1	A_1	A_3	new A_1
No. of patients	22	58	88	133
Age range	1y0m–8y9m	1y0m–13y8m	1y0m–11y11m	1y0m–18y5m
Mean age	3y11m	4y7m	2y8m	4y5m
Male/female	14/8	32/26	54/34	83/50
No. of pts (%) with radical surgery*	14 (63.6)	52 (89.7)	71 (80.7)	99 (74.4)
No. of CR (%)	9 (40.9)	43 (74.1)	63 (71.6)	93 (69.9)
No. of PR (%)	9 (40.9)	12 (20.7)	21 (23.9)	31 (23.3)
No. of SD (%)	1 (4.5)	2 (3.4)	2 (2.3)	6 (4.5)
No. of PD (%)	3 (13.6)	1 (1.7)	2 (2.3)	3 (2.2)
No. of pts with ABMT/PBSCT (%)	7 (31.8)	14 (24.1)	63 (71.6)	71 (53.4)
No. of pts receiving conditioning regimens P, Q, R, and S (see Table 2)	P: 5 R: 2	P: 7 R: 6 S: 1	P: 23 Q: 12 R: 24 S: 4	P: 19 Q: 8 R: 39 S: 5
5-year relapse-free survival (%)	23.2**¶	33.3**	36.0¶	32.2
5-year overall survival (%)	32.8#	42.8	34.0#	38.9

*Total resection plus subtotal resection.
†$P = 0.029$.
‡$P = 0.003$.
§$P = 0.022$.
CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease after WHO (14).

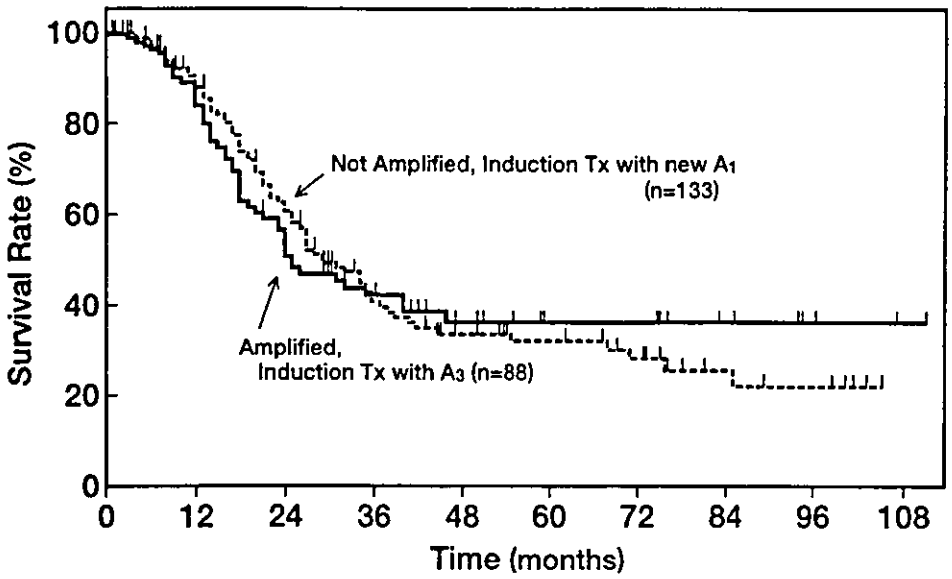

FIG. 4. Relapse-free survival rates for stage 4 patients older than 12 months with and without *MYCN* amplification treated after March 1991 with induction chemotherapy regimens A_3 and new A_1, respectively ($P = 0.849$). Time was measured from the start of treatment.

pan, but the incidence of advanced neuroblastoma did not decrease much, and referrals to participating institutions had increased during the 15 years of the studies.

Seeger et al.'s (1) reported 9-month progression-free survival rates were 61%, 47% and 0% in stage IV (15) neuroblastoma patients whose tumors had one, three, and 10 or more copies of *MYCN*, respectively ($P < 0.0001$). Their results were based on chemotherapeutic regimens in use more than 15 years ago, and the treatment of high-risk neuroblastoma has recently improved in the United States (6) and Japan (7). Thirteen (24%) of 55 stage 4 patients with amplified *MYCN* survived disease-free for more than 66 months in a study by Kawa et al. (7). Matthay et al. (6) achieved significantly improved survival rates and reported that the 3-year relapse-free survival rates after the first randomization were 27% for all patients, 34 ± 4% for those undergoing ABMT, and 22 ± 4% for those receiving continuation chemotherapy. They used a single protocol for patients with stage 4 neuroblastoma with and without *MYCN* amplification and concluded that there was still a difference in survival between these two groups ($P = 0.03$). This indicates that *MYCN* amplification affects survival in

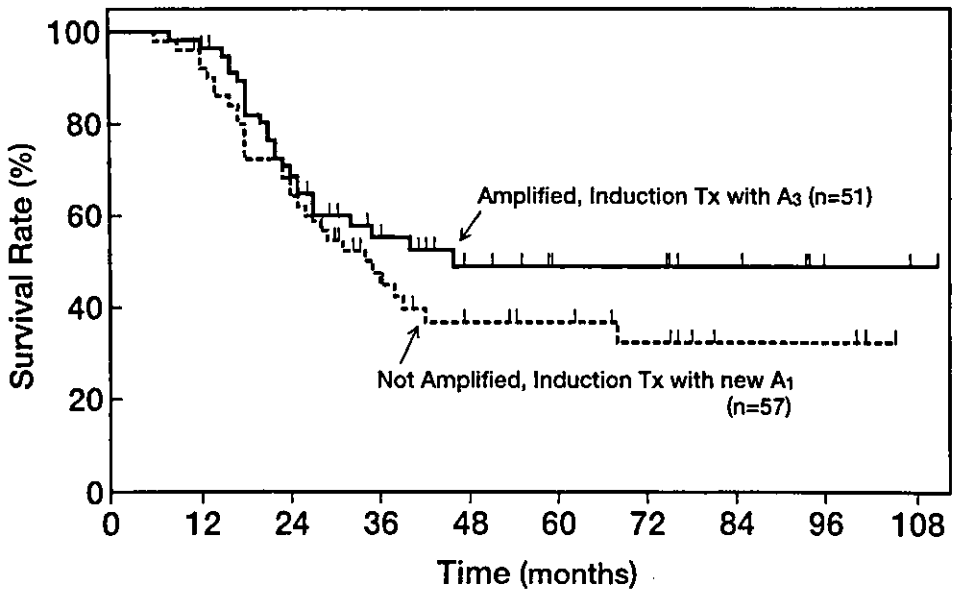

FIG. 5. Relapse-free survival rates for stage 4 patients older than 12 months with and without *MYCN* amplification treated with autologous bone marrow transplantation or peripheral blood stem cell transplantation when in complete remission ($P = 0.585$). Time was measured from the second randomization.

therapy, including ABMT/PBSCT. No other group has used different protocols for patients with stage 4 neuroblastoma based on *MYCN* amplification status. Improvement in the clinical results of stage 4 patients with *MYCN* amplification after high-dose chemotherapy and ABMT/PBSCT strongly suggests that further basic studies are needed to determine the effects of cyclophosphamide or other agents on the molecular mechanism of progression of tumors with *MYCN* amplification.

The prognosis for patients with stage 4 neuroblastoma without *MYCN* amplification remains poor (13,16,17). There are a few reasons why. One reason is that current assessments of *MYCN* amplification status are based on the Southern blot technique as originally performed in this tumor (1,18). However, two reports on neuroblastoma cell lines showed that *MYCN*-nonamplified tumors sometimes express *MYCN* mRNA and MYCN protein, presumably because of the prolonged half-life of this protein in some tumors (19,20). Strictly speaking, assessment of *MYCN* amplification and expression status should be based not only on Southern blotting but also on demonstration of the presence of MYCN protein. One report (21) indicated that expression of MYCN protein without amplification might occur only in a few patients with stage 4 neuroblastoma. There may be other, unknown reasons for this. For example, the implication of 3 to 9 copies of *MYCN* in stage 4 neuroblastoma should also be taken into account. Alternatively, other unknown or known genetic factors such as abnormalities on chromosomes 2q, 9q, 11q, 14q, and 17q may play a role (22–26). The results in stage 4 *MYCN*-nonamplified patients are so dismal that we started in June 1998 to treat *MYCN*-nonamplified stage 4 patients with regimen A_3. However, *MYCN*-amplified stage 4 patients are now being treated either with regimen A_3 or regimen D (ifosfamide 2,800 mg/m^2 per day and etoposide 120 mg/m^2 per day on days 1 through 5).

The current study showed that intensified chemotherapy improved relapse-free survival rates for children older than 12 months with stage 4 *MYCN*-amplified neuroblastoma. The 5-year relapse-free survival rate increased from 23.2% to 36.0%. In the Kaplan–Meier survival curves (Figs. 3,4), high-dose chemotherapy appears to have abolished the effects of *MYCN* amplification. Nevertheless, the clinical results remain unsatisfactory. It is possible that new therapies, such as regimen A_3, merely delay relapse rather than contribute to a truly improved survival rate. Further innovative treatment of patients with stage 4 *MYCN*-amplified neuroblastoma is awaited, as are additional basic studies on the mechanism by which the progression of *MYCN*-amplified stage 4 neuroblastoma is altered by high-dose chemotherapy.

Acknowledgments: The authors thank Drs. A. Hayashi, Y. Toyoda, M. Kobayashi, and S. Suenobu for special collaboration, and Ms. Cynthia Yenches for editorial assistance.

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