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IRINOTECAN FOR CHILDREN WITH RELAPSED SOLID TUMORS

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□ *Irinotecan is expected to become a new drug for childhood solid tumors. Sixteen children with relapsed solid tumors received irinotecan 180 mg/m^2/day for 3 consecutive days, repeated once after 25 days off. Their original tumors were neuroblastoma in 7, rhabdomyosarcoma in 3, nephroblastoma and undifferentiated sarcoma in 2 each, and primitive neuroectodermal tumor and leiomyosarcoma in 1 each. The average age at trials was 6 years. Partial response was achieved in 5 (31.3%) (neuroblastoma, rhabdomyosarcoma, nephroblastoma, undifferentiated sarcoma, and leiomyosarcoma), and decrease in tumor marker in the other 2. Irinotecan appears promising, and could become included in the first-line treatment.*

Keywords clinical trials, irinotecan, nephroblastoma, neuroblastoma, rhabdomyosarcoma

Camptothecin (CPT), an alkaloid with a novel ring structure, was first isolated from the Chinese tree *Camptotheca acuminate*, and 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]. Irinotecan as well as topotecan [2] inhibits DNA topoisomerase I, which is an essential nuclear enzyme that relaxes torsionally strained duplex DNA, enabling replication and transcription [3]. Irinotecan has been reported to be effective against various human malignancies, including 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 [4–13]. Four phase I trials of irinotecan in children were conducted in the United States, France, and Japan [14–17], and investigators in these four groups have proposed their own appropriate drug doses and administration schedules for the use in phase II trials.

A variety of approaches to the treatment of advanced pediatric solid tumors such as neuroblastoma have been employed, and the clinical results have improved in recent years [18, 19]. Nevertheless, the prognosis is dismal, particularly once patients with such tumor relapse after myeloablative therapy followed by stem cell transplantation, and it appears appropriate to investigate the activity of new agents such as irinotecan for these patients [3]. We therefore started clinical trials of the use of irinotecan in our patients who relapsed. We were also strongly encouraged to do so with our promising results in preclinical studies of irinotecan, which were conducted against several pediatric solid tumor xenograft models [20–22].

While other investigators [23] are reporting mediocre phase II results, much better clinical results were obtained in our trials of irinotecan in Japan, prompting us to write this article.

PATIENTS AND METHODS

This study was conducted by selected members of the Study Group of Japan for Treatment of Advanced Neuroblastoma [19]. Patients with

histologically confirmed, measurable solid tumors that were deemed to be treatment failures on conventional treatment (relapsed or refractory) were eligible for this trial. Other eligibility criteria included a life expectancy of at least 3 months, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, at least 4 weeks since and recovery from the toxic effects of previous chemo- and radiotherapy, hemoglobin concentration ≥8.0 g/dL, granulocyte count ≥1000/mm^3, and platelet count ≥50,000/mm^3. Other requirements included normal liver function (total 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, according to the recommendation of the Society of Japanese Pharmacopoeia [24]. Written informed consent for participation was obtained from all patients or their guardians, and the study protocol approved by the institutional review boards of participating institutions.

Patients were evaluated for nonhematopoietic toxicity with the ECOG common toxicity criteria [25] and for hematopoietic toxicity with a table of hematopoietic toxicities [17] modified by the current study group from the ECOG criteria (Table 1). The idea for 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 1000/mm^3 and 50,000/mm^3, respectively. The tumors were measured on the longest diameter, and the response was evaluated by the guidelines developed by Therasse and others [26]. Responses such as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) were centrally reviewed, evaluated at 4 weeks after

TABLE 1 Nonhematopoietic Toxicities of the ECOG Common Toxicity Criteria [25] and Hematopoietic Toxicities Modified from the ECOG Common Toxicity Criteria for the Present Study [17]

	Grade				
	0	1	2	3	4
Serum aspartate transaminase (× nL)	<1.5	1.5–2	2.1–5	>5	—
Alkaline phosphatase (× nL)	<1.5	1.5–2	2.1–5	>5	—
Bilirubin (× nL)	<1.5	1.5–2	2.1–5	>5	—
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 (×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

TABLE 2 Summary of the Cases Studied

Patient	Age (years)	Sex	Disease	Clinical response
1	12	F	Leiomyosarcoma	PR
2	9	M	Neuroblastoma	SD
3	4	M	Neuroblastoma	PD
4	11	F	PNET	PD
5	8	M	Neuroblastoma	SD*
6	9	F	Neuroblastoma	SD*
7	4	F	Neuroblastoma	PR
8	1	F	Undifferentiated sarcoma	PR
9	2	F	Nephroblastoma	PR
10	5	F	Nephroblastoma	PD
11	5	F	Neuroblastoma	SD
12	3	F	Rhabdomyosarcoma	PD
13	6	M	Rhabdomyosarcoma	PR
14	11	M	Undifferentiated sarcoma	PD
15	1	M	Neuroblastoma	SD
16	6	F	Rhabdomyosarcoma	PD

Note. Dose, 180 mg/m^2/day for 3 consecutive days. PNET, primitive neuroectodermal tumor; SD*, stable disease but with transient decrease in tumor marker levels.

the 2nd course of treatment, and 4 weeks was a minimum time period for confirmation of response [26].

The expected response (CR + PR) ratio was set to 20%.

RESULTS

Results of the study, performed from June 2001 to November 2004, are shown in Tables 2 and 3. The original tumors were neuroblastoma in 7, rhabdomyosarcoma in 3, nephroblastoma and undifferentiated sarcoma in 2 each, and primitive neuroectodermal tumor (PNET) and leiomyosarcoma in 1 each. One patient had a refractory tumor, and the remaining 15 had relapsed tumors. Age of the patients at the start of trials ranged from 1 year

TABLE 3 Adverse Effects Observed in 32 Courses of Irinotecan Treatment for 16 Patients

	Grade 0	Grade 1	Grade 2	*Grade 3*	*Grade 4*
Myelosuppression					
White blood cell count	0	1	8	22	*1*
Granulocyte count	0	1	5	13	*13*
Platelet count	5	6	8	11	*2*
Gastrointestinal tract					
Diarrhea	3	3	15	*4*	*7*
Vomiting	1	4	18	8	1

Note. Italic type indicates severe adverse effects.

to 12 years, and the average age was 6 years. Thirty-two treatment courses were administered to these 16 patients. All patients were given irinotecan 180 mg/m^2/day by 120-min drip infusion for 3 consecutive days repeated once after 25 days off. A partial response (PR) was achieved in 5 (neuroblastoma, rhabdomyosarcoma, nephroblastoma, undifferentiated sarcoma, and leiomyosarcoma), and transient decrease in tumor marker levels was observed in the other 2 patients with neuroblastoma. Therefore, PR was observed in 31.3% of the relapsed/refractory patients with the current administration schedule of irinotecan, which is different from the results of the other investigators. The response (CR + PR) ratio was much higher than expected, and exceeded the response rate of 21.1% by Cosetti et al. [27].

Of 7 children with relapsed neuroblastoma, PR was achieved in 1, SD but with transient decrease in urinary tumor marker levels in 2, SD alone in 3, and PD in 1. One of 3 patients with rhabdomyosarcoma had a PR, but the response was PD in the remaining 2. Similarly, one of 2 patients with nephroblastoma showed PR, but the response was PD in the remaining one, and 1 of 2 patients with undifferentiated sarcoma showed a PR, but the response was PD in the remaining 1.

Grade 3 suppression of white blood cell count was observed in 69% of the patient courses, grade 3 to 4 suppression of granulocyte count in 81%, and grade 3 to 4 suppression of platelet count in 41%, whereas diarrhea of grade 3 or greater was seen in 34% (Table 3), but these side effects were all managed well using routine clinical methods, with recombinant human granulocyte colony-stimulating factor (rhG-CSF), oral or intravenous antibiotics, and/or central line fluid administration. Prophylactic administration of loperamide were not recommended, but in patients who developed diarrhea, loperamide and/or antibiotics were given with subsequent courses to decrease gastrointestinal toxicity, according to the judgment of each investigator.

DISCUSSION

Among the camptothecin derivatives, topotecan and irinotecan are most widely used in clinical trials [3]. As to the use in children, irinotecan appears to be promising in the treatment of such tumors as rhabdomyosarcoma, neuroblastoma, and desmoplastic round blue cell tumor [13–17]. The recommended dose and administration schedule of irinotecan differ among researchers [14–17]. Furman and coworkers [14] recommend administration of irinotecan 20 mg/m^2/day for 5 consecutive days, repeated once after 2 days off (10 days' administration altogether) based on their results of irinotecan experiments in an in vivo system. On the other hand, Vassal and coworkers [16] reported that the maximum tolerated dose (MTD) of irinotecan for children was 600 mg/m^2 when given as a 120-min intravenous infusion every 21 days. Mugishima and others from Japan [17] determined that the

TABLE 4 Recommended Administration Schedules for Phase II Trials of Irinotecans and Some Results of Phase II Trials

Authors	Results of phase I (MTD) and recommended schedules for phase II	Results of phase II trials
Mugishima et al. [17] (Japan)	180 mg/m^2/day for 3 consecutive days; repeated every 4 weeks	31.3% response rate; 5 PR in 16 patients (present report)
Furman et al. [14] (USA)	20 mg/m^2/day for 5 consecutive days; repeated after 2 days off; repeated every 4 weeks	21.1% response rate; 2 CR/2 PR in 19 patients (Cosetti et al. [27])
Blaney et al. [15] (USA)	50 mg/m^2/day , daily for 5 days, beginning every 3 weeks	Ongoing
Vassal et al. [16] (France)	600 mg/m^2/day; repeated every 3 weeks	Disappointing in neuroblastoma (Vassal et al. [23])

Note. MTD, maximum tolerated dose.

MTD of irinotecan for children should be between 160 and 180 mg/m^2/day administered over 3 consecutive days, repeated once after 25 days off. These MTDs are currently recommended for phase II trials in the respective groups (Table 4), but these four regimens have both merits and demerits [14–17]. Cosetti et al. [27] reported 4 objective responses (2 CR and 2 PR) (21.1%) in 19 evaluable patients treated on the administration schedules developed by Furman et al. [14], but the authors' response rate of 31.3% (5 PR in 16 cases) was superior to their results [27].

Although it might be too premature to refer to the relationship between the nature of the tumor and the efficacy of irinotecan, Cosetti et al. [27] already observed CP + PR in 6 of 7 patients with relapsed rhabdomyosarcoma on the schedule of Furman et al. [14], and this observation coincides with our results in rhabdomyosarcoma with 1 PR in 3 cases, although they used a different mode of administration. On the other hand, the use of irinotecan for neuroblastoma in a single day by Vassal et al. [23] met with disappointing results with no clinically useful activity in their phase II trials. They noted that since the majority of children had received very intensive induction treatments and retinoids it made it unlikely that a single agent in a phase II setting would demonstrate activity. They considered that they needed to evaluate neuroblastoma in a different setting in the future to prevent clinically important agents from being overlooked [23]. The authors observed one PR and two cases of transient decrease in tumor marker levels in 7 relapsed neuroblastomas, and the patient who showed PR (Table 2) at the evaluation is now disease-free without treatment for 46 months since the second remission [28].

It is also hard to conclude the relationship between the administration schedule and the effectiveness of irinotecan. Protracted per oral use of irinotecan might be recommended because of its consistent effectiveness [22, 29], but the use of irinotecan over 12 days could be somewhat burdensome for patients and clinicians. Vassal et al. prefer the use of it in a single

day [16, 23], and the Japanese group considers that the administration over 3 consecutive days may have an advantage over the others, because they already confirmed protracted plasma concentrations of irinotecan with their 3-day administration schedule [17]. The administration schedule for 5 days developed by Blaney et al. [15] was used widely in the Children's Oncology Group, but the results are not published yet. As a single, independent experience, Rosoff and Bayliff [13] administered irinotecan 50 mg/m^2/day for 5 days every 3–4 weeks in 2 children with desmoplastic round blue cell tumors and saw significant responses. In the ongoing Children's Oncology Group study, ARST 0121, in children with relapsed or progressive rhabdomyosarcoma, patients are randomized to receive irinotecan either on days 1–5 or on days 1–5 and 8–12, though this is not a single-agent study [30].

As the effectiveness as well as the toxicity profile of irinotecan differs depending on the schedule, careful clinical care of the patients will be mandatory. The use of rhG-CSF is advisable to prevent granulocytopenia following the administration of irinotecan 180 mg/m^2/day for 3 days.

There have been significant advances in the treatment of advanced neuroblastoma and rhabdomyosarcoma in recent years [18, 19, 31, 32], but the clinical results are still poor, especially once the tumor relapses. The responses reported by us and by others [27] with various tumors suggest that irinotecan is promising, should be explored further in late phase II trials, and might be included as an active agent in the first-line treatment of pediatric solid tumors with poor prognosis.

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神経芽腫

neuroblastoma

Key words: 神経芽腫

定義・概念 神経芽腫の用語は 1910 年に Wright が初めて neuroblastoma という用語を使用したことに始まる。神経芽腫は胎生期の神経堤（neural crest）を起源とする神経芽細胞が成熟分化せずに腫瘍化したものと考えられ，副腎髄質および交感神経系組織に発生する胎児性腫瘍である。神経芽腫の名称は，組織学的に狭義の神経芽腫（neuroblastoma）と神経節芽腫（ganglioneuroblastoma）および神経節腫（ganglioneuroma）の 3 種類の腫瘍を総称するものであり，神経芽腫群腫瘍（pNTs: peripheral neuroblastic tumors）と呼称する場合もある。

発生頻度は小児悪性固形腫瘍の中で脳腫瘍に次いで多い。アメリカでは 7,000 人出生に対し 1 人の割合で発生し，年間約 600〜650 人の発生頻度がある。日本の小児癌登録事業は整備中であり神経芽腫の正確な発生頻度は把握されていないが，（財）がんの子供を守る会小児がん全国登録委員会の小児悪性新生物・全国登録委員会報告事業によると年間約 200 人弱の登録がある。これらは神経芽腫マススクリーニング事業によって無症候性に発見された症例を含むため，症候性に発見される症例の発生頻度は不明である。神経芽腫の発生は 1 歳以下のピークと 3〜4 歳のピークの 2 峰が存在する。

病態生理 神経芽腫の好発部位は副腎が 65％で最も多く，その他は頸部，後縦隔，後腹膜，骨盤腔などの交感神経節である。左右差は 3：2 の割合で左に多い。転移は骨，骨髄，肝，リンパ節，皮膚，眼窩などに認められる。脳あるいは肺転移の報告もある。神経芽腫の多くでは血清カテコラミン（ドパミン，エピネフリン，ノルエピネフリン）を産生し，その尿中代謝産物であるバニルマンデル酸（VMA）とホモバニリン酸（HVA）などが上昇する。狭義の神経芽腫はクロマチンに富む核と乏しい細胞質からなる小円形腫瘍細胞と，わずかな神経線維とからなり，その間に間質細胞が存在する。神経節芽腫は未分化な神経芽細胞と分化した神経節細胞が混在するものである。Beckwith らの研究から胎生期には多数の *in situ* neuroblastoma が存在し，その大多数が自然に成熟または消退し，残りのごく一部が神経芽腫になるものと推定されている。マススクリーニング症例の検討から，神経芽腫のうちあるものは自然退縮や分化成熟し予後良好であることが判明した。しかし 1 歳以上で診断される症例は病期が進行し，また生物学的予後因子も不良なものをもつことが多く，治療抵抗性で予後不良な例が多い（進行例）。両者を明確に区別する指標はいまだ明らかではない。これまでの研究から，年齢，stage，病理組織学的分類，*MYCN*，DNA ploidy，*trkA* などが予後と関連していると考えられている。

臨床症状 臨床症状には原発部位の腫瘤による症状と転移による症状があり，年齢と原発部位，病期により異なる。乳児期早期の症例は多くが 4S 期で，びまん性肝転移による腹部膨満症状とそれによる胸部圧迫のための呼吸器症状を認めることが多い。乳児期のマススクリーニング発見例は一般的に無症状である。進行例には腹部膨満，食欲不振，発熱などのほかに遠隔転移の症状としての顔面蒼白，貧血，眼球突出，眼瞼出血，骨痛，関節痛，跛行などが認められることが多いが，発熱のみの場合や偶然の腹部腫瘤触知による発見まで無症状のこともある。特殊な症状として，Horner 症候群や opsomyoclonus，小脳性運動失調あるいは脊椎管内への腫瘍進展による神経麻痺，腫瘍から産生されるカテコラミンによる異常な発汗や高血圧，血管作動性腸ペプチド産生による水様性下痢などがみられることがある。

診断 診断は原発腫瘍または転移巣の開創生検を行い光学顕微鏡検査により病理組織学的に確定診断する。あるいは，骨髄検査で腫瘍細胞の転移が確認され，かつ尿中 VMA，HVA が明らかに高値である場合は，原発腫瘍の組織学的検討を行わずに神経芽腫と診断してよい。しかしながら原則として原発腫瘍の開創生検を行い病理組織学的な診断を行うべきである。針生検による病理診断は正確な診断に至ることが困難な場合があり，また治療方針決定に必要な生物学的予後因子の検索ができないことがあり，神経芽腫以外の固形腫瘍の場合も含め勧められない。*MYCN* コピー数や DNA ploidy などの分子生物学的予後因子の検索を行うことがリスク分類による治療方針の決定には必要である。病理組織学的分類は International Neuroblastoma Pathology Classification（INPC）が国際的に一般的である。これは神経芽腫細胞の形態に，診断時年齢，Schwann 細胞の発達（stroma），神経細胞の分化程度（differentiation），Mitosis-karyorrhexis index（MKI）を加味し，組織型としては neuroblastoma, ganglioneuroblastoma: intermixed, ganglioneuroma, ganglioneuroblastoma: nodular の 4 型に分類し，さらに予後のグループとして favorable histology group と unfavorable histology group に分類するものである。

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表1 神経芽細胞腫国際病期分類（International Neuroblastoma Staging System/INSS）

病期	定義
1	限局性腫瘍で，肉眼的に完全切除。組織学的な腫瘍残存は不問。同側のリンパ節に組織学的な転移を認めない（原発腫瘍に接し，一緒に切除されたリンパ節転移はあってもよい）。
2A	限局性腫瘍で，肉眼的に不完全切除。原発腫瘍に接しない同側リンパ節に組織学的に転移を認めない。
2B	限局性腫瘍で，肉眼的に完全または不完全切除。原発腫瘍に接しない同側リンパ節に組織学的に転移を認める。対側のリンパ節に転移を認めない。
3	切除不能の片側性腫瘍で，正中線（対側椎体縁）を越えて浸潤。同側の局所リンパ節の転移は不問。または，片側発生の限局性腫瘍で対側リンパ節転移を認める。または，正中発生の腫瘍で椎体縁を越えた両側浸潤（切除不能）か，両側リンパ節転移を認める。
4	いかなる原発腫瘍であるかにかかわらず，遠隔リンパ節，および/または，骨，骨髄，肝，皮膚，ほかの臓器に播種している（4S は除く）。
4S	限局性腫瘍（病期 1，2A，2B）で，播種は皮膚，および/または，肝，骨髄に限られる（1 歳未満の患者のみ）。骨髄中の腫瘍細胞は有核細胞の 10%未満で，それ以上は病期 4 である。MIBG シンチが行われるならば骨髄への集積は陰性。

（Brodeur GM, et al: J Clin Oncol 11 (8): 1466-1477, 1993）

表2 効果判定規準（International Neuroblastoma Response Criteria/INRC）

評価	原発巣	転移巣
CR (complete response)	腫瘍なし	腫瘍なし カテコールアミン代謝産物正常化
VGPR (very good partial response)	90～99%縮小	腫瘍なし カテコールアミン代謝産物正常化 骨シンチでの集積は残存していてもよい （MIBG シンチは陰性化していなければならない）
PR (partial response)	50%以上縮小	測定可能病変が 50%以上縮小 骨転移の病変数が 50%以上減少 骨髄転移の病変数は 0～1 か所 （MIBG シンチでの集積は残存していてもよい）
MR (mixed response)	新病変の出現なし 原発巣および転移巣の測定可能病変において 50%以上縮小する病変を認める 同時にほかの病変は 50%未満の縮小や 25%未満の増大を示す	
NR (no response)	新病変の出現なし 原発巣および転移巣の測定可能病変は，50%未満の縮小や 25%未満の増大を示す	
PD (progressive disease)	新病変の出現 あるいは原発巣および転移巣の測定可能病変において 25%以上の増大を示す病変を認める もしくは骨髄の転移病変の新たな出現	

CR，VGPR，PR，MR，NR については定義に述べられたすべての要件を満たしていることが必要である。
PD に関しては定義に述べられたいずれかの要件を満たした状態である。

（Brodeur GM, et al: J Clin Oncol 11 (8): 1466-1477, 1993）

病期分類 これまで病期分類は日本小児外科学会悪性腫瘍分類，Evans sysytem，St. Jude Children's Research Hospital and POG classification などが使用されてきたが，現在は神経芽細胞腫国際病期分類（International Neuroblastoma Staging System/INSS）（**表1**）が使用される。病期分類には初診時での原発腫瘍の拡がり，リンパ節転移，肝転移，あるいは神経芽腫の好発部位である交感神経の経路に沿った部位への転移の把握が必要であり，これには全身の X 線 CT や MRI が必要である。さらに骨および骨髄転移の検索が必須で，^{123}I metaiodobenzylguanidine（MIBG）シンチグラフィおよび ^{99m}Tc 骨シンチグラフィが必要である。骨髄転移の検索には，治

療効果の判定として International Neuroblastoma Response Criteria（INRC）（**表 2**）を用いる場合には左右 2 か所の腸骨での骨髄穿刺吸引検査と左右 2 か所の骨髄生検が必要とされている。

リスク分類 神経芽腫は年齢，病期，病理学的特徴，分子生物学的特徴などにより著しく予後が異なる。このため治療選択の基準として，病期分類にさらにいくつかの予後因子を組み合わせたリスク分類の必要性が提唱されている。リスクは予後との関連により低リスク群，中間リスク群，高リスク群に分類することが一般的である。これまでは日米欧で独自のリスク分類が提案されてきたが，現在は国際的な統一分類の開発が企画され討議されている。代表的なリスク分類である COG のリスク分類では，年齢と INSS 病期分類，INPC 組織分類，*MYCN* 増幅の有無および DNA index により**表 3** のように分類している。

鑑別診断 悪性リンパ腫，Ewing 肉腫ファミリー腫瘍，横紋筋肉腫と神経芽腫は，HE 染色による形態判断では鑑別が困難な場合があり，これらの疾患を総称して小円形細胞腫瘍と呼んでいる。これらの鑑別には生検あるいは摘出組織を利用して免疫染色や電子顕微鏡検査あるいは分子生物学的検査を施行する必要がある。また，いわゆる腫瘍マーカーとしての血清カテコールアミンや VMA, HVA などを確認することが有効である。NSE は小円形細胞腫瘍ではいずれの腫瘍でも上昇することがある。また原発部位が腎近傍の場合には腎芽腫などの腎原発腫瘍の鑑別も必要である。

予後 予後を規定する最も重要な因子は適切な治療法の開発である。このため現在では上述のようにリスク分類による治療法の選択について真剣に討議されている。リスク分類はいまだ議論の途上ではあるが，今後治療成績の集積により改善していくものと考えられる。一般的には，1 歳以上の患者は予後不良と考えられている。また stage 4 あるいは *MYCN* が増幅している進行神経芽腫の予後は，骨髄破壊的大量化学療法を行っても 3 年無増悪生存率は 20～40％台にすぎない。主な再発形式は骨あるいは骨髄再発である。現在アメリカでは COG のリスク分類に基づき 3 年全生存率を低リスク群で 90％超，中間リスク群で 70～90％，高リスク群で 30％超と推測している。日本全体としての治療成績は明らかではない。

治療方針 神経芽腫治療は以前よりリスクに基づいた治療戦略が行われてきた。現在の COG の治療方針は低リスク群では外科切除後経過観察，中間リスク群では外科切除と通常の化学療法，高リスク群では集学的治療が必要となり，外科切除に加え積極的な化学療法と大量化学療法＋自家造血細胞移植および放射線療法である。日本においても以前よりほぼ同様の治療方針が採られている。

高リスクに対する治療 現在日米欧で行われている高リスク群の治療戦略は，診断時には原発巣が全摘出できる症例がほとんどないこと，および骨，骨髄転移例が多く速やかな全身化学療法の開始が必要であることから，初回手術は診断目的の生検にとどめられ，寛解導入療法としての化学療法を数コース行った後，局所療法として外科切除術および局所放射線療法を組み合わせた治療を行い，その後強化した化学療法あるいは骨髄破壊的大量化学療法による地固め療法を行うものである。寛解導入療法としては，シスプラチン，トポイソメラーゼ II 阻害剤，アントラサイクリン系薬剤，ビンクリスチン，シクロホスファミドのうちから 3～5 種類の薬剤を組み合わせた多剤併用療法が一般的に行われている。治療回数は間に外科手術や放射線治療を挟む形で 5～7 回程度行われる計画が多い。外科療法の施行時期と腫瘍の切除度合については議論が分かれており，結論がでていない。神経芽腫はいまだ治療法開発段階であり，今後は整備された前方視的な多施設共同臨床研究に従い治療が行われることが望ましいと考えられる。

表 3 COG リスク分類

低リスク
1．患者の年齢を問わず INSS 1 期
2．1 歳未満の INSS 2A 期および 2B 期
3．1 歳以上で，FHG の INSS 2A 期および 2B 期
4．1 歳以上で，*N-MYC* 増幅なしの INSS 2A 期および 2B 期
5．1 歳未満で，*N-MYC* 増幅なし，かつ FHG，かつ高二倍体 DNA である INSS 4S 期

中間リスク
1．1 歳未満で，*N-MYC* 増幅なしの INSS 3 期
2．1 歳以上で，*N-MYC* 増幅なし，かつ FHG の INSS 3 期
3．1 歳未満で，*N-MYC* 増幅なしの INSS 4 期
4．1 歳未満で，*N-MYC* 増幅なし，かつ二倍体に近い DNA の INSS 4S 期
5．1 歳未満で，*N-MYC* 増幅なし，かつ UFHG の INSS 4S 期

高リスク
1．1 歳以上で，*N-MYC* 増幅あり，かつ UFHG の INSS 2A 期および 2B 期
2．患者の年齢を問わず，*N-MYC* 増幅ありの INSS 3 期
3．1 歳以上で，UFHG の INSS 3 期
4．1 歳未満で，*N-MYC* 増幅ありの INSS 4 期
5．1 歳以上の INSS 4 期
6．1 歳未満で，*N-MYC* 増幅ありの INSS 4S 期

FHG: INPC で favorable histology group，UFHG: INPC で unfavorable histology group
記載のない項目は不問である。
（Castleberry RP: Eur J Cancer 33: 1430-1437, 1997）

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MYCN gene amplification is a powerful prognostic factor even in infantile neuroblastoma detected by mass screening

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MYCN is the most powerful prognostic factor in cases of older children. However, how *MYCN* is related to the prognosis of infantile cases is not clear. A mass screening program was carried out by measuring urinary catecholamine metabolites (VMA and HVA) from 6-month-old infants. Of 2084 cases detected by the screening program, *MYCN* amplification (MNA) was examined by Southern blot analyses in 1533 cases from 1987 to 2000. Of the 1533 cases examined, 1500 (97.8%) showed no MNA, 20 cases (1.3%) showed MNA from three to nine copies, and 13 (0.8%) cases showed more than 10 copies. The 4-year overall survival rates of these three groups (99, 89 and 53%, respectively) were significantly different ($P<0.001$), indicating that *MYCN* copy number correlates with the prognosis. Cases with MNA more than 10 copies were more advanced than those without amplification (stage III, IV *vs* I, II, IVs; $P<0.001$). Patients with MNA more than 10 copies had significantly higher serum levels of neuron-specific-enolase (NSE) and ferritin than non-amplified patients ($P=0.049$, $P=0.025$, respectively). *MYCN* amplification was strongly correlated with a poor prognosis in infantile neuroblastoma cases. Therefore, for the selection of appropriate treatment, an accurate determination of MNA is indispensable.

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Keywords: neuroblastoma; infant; *MYCN*; mass screening

Neuroblastoma (NB) is characterized by heterogeneous tumours, some of which regress spontaneously while others proliferate and progress (D'angio *et al*, 1971; Evans *et al*, 1971; Look *et al*, 1991). The prognosis for NB in infants is much more favourable than it is in older children. In 1973, the Mass Screening Program for Neuroblastoma (MSPN) was commenced for the early detection of NB in children living in Kyoto, Japan. A nationwide MSPN for 6-month-old infants began in 1985 (Sawada *et al*, 1984). The latter MSPN revealed incidences of infantile NB in the early stages and good biological prognostic factors of tumours increased (Hachitanda *et al*, 1994; Sawada *et al*, 1998). However, it has been argued that MSPN might result in the overdiagnosis of tumours, because some of the tumours might spontaneously regress (Yamamoto *et al*, 2002; Honjyo *et al*, 2003). And, the researcher has concluded that the screening was ineffective, because clustered randomized trials have not shown that screening led to a significant reduction in mortality rate from NB (Woods *et al*, 2002; Kerbl *et al*, 2003). Consequently, criticism has arisen that MSPN might detect only redundant tumours with good prognostic factors. Actually, the prognosis in most NB cases detected by MSPN has proved to be good. However, some cases detected by MSPN have poor prognostic factors resulting in relapsed disease (Kusafuka *et al*, 1995). Moreover, there are reports that the good prognosis has been obtained by early treatment in infantile NB cases with poor prognostic factors (Kusafuka *et al*, 1995; Tanaka *et al*, 1998).

Although *MYCN* is well known to be the most powerful prognostic factor in noninfantile cases of NB, how *MYCN* is related to the prognosis of infantile cases, especially those discovered by MSPN, is not clear. Therefore, we assessed *MYCN* amplification (MNA) in infantile cases. If the prognoses of infantile NB cases detected by mass screening and MNA correlate strongly, it is necessary to evaluate MNA to decide on the appropriate treatment for these cases.

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[9] For more details of Committee of Neuroblastoma in the Japanese Society of Pediatric Oncology see Appendix A1
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PATIENTS AND METHODS

Analysis of urine catecholamine

Kits for screening children for urinary chatecholamines were provided to the parents at public health centres throughout Japan

when they brought their child in for a health checkup at 3 months of age. Urine was collected by parents at home and sent to screening centres by mail. Urine samples were assayed for vanillylmandelic acid (VMA) and homovanillic acid (HVA) by high-performance liquid chromatography (HPLC). When children's urinary levels of either VMA or HVA were >2.5 s.d. above normal, the child was given clinical examinations for NB at a hospital. The normal range was based on levels in healthy infants of an age-matched (Sawada, 1988).

Patient population

Between April 1987 and March 2000, the population of the target infants was 17 139 975. Of this number, 14 496 103 (84.6%) were screened for elevated catecholamine levels. Of this number, 2084 children were diagnosed as having NB based on urinary catecholamine levels and were registered with the Committee of Neuroblastoma in the Japanese Society of Pediatric Oncology.

Staging The extent of the disease was evaluated according to the Evans's stage classification (Evans *et al*, 1971). The International Staging System (INSS) (Brodeur *et al*, 1993) had not yet been introduced when the MSPN began.

Biological features The prognosis and clinical features of these cases were evaluated on the basis of the MNA. *MYCN* amplification in tumour samples was detected using a Southern blot analysis with *MYCN* second-exon probe according to standard procedures (Brodeur *et al*, 1984). Although cases with 10 copies or more of the *MYCN* gene are classified into the high-risk group in Japan (Kaneko *et al*, 2002), in this study the *MYCN* gene was considered amplified if there were more than three copies.

Registry

The hospitals reported the cases to the registration centre within 2 years of the findings of elevated catecholamine levels in the screening process. The hospital reported the outcome of each case 5 years after the initial diagnosis of NB. However, the outcome of the cases diagnosed between 1999 and 2000 has been 2 years since the appearance of disease.

Statistical analysis

The Kaplan–Meier product limit method was used to estimate the event-free survival (EFS) and overall survival (OS) from the time of diagnosis of NB. The log-rank test was performed to compare the OS probabilities between subgroups of patients. The differences between dichotomous variables were analysed by χ^2 test when samples were of sufficient size. The two-tailed *t*-test was carried out to compare the distributions of continuous variables. A two-tailed *P*-value of <0.05 was considered to indicate statistical significance.

RESULTS

Of 1533 infants with elevated urinary catecholamine levels that were examined for MNA, 33 (2.2%) had tumours with MNA. Of these 33 cases, 20 had *MNA* values from three to nine copies of the *MYCN* gene (Table 1). Seventy-seven percent of cases with no MNA had early stage (stages I, II and IVs) tumours. Thirteen cases had more than 10 copies. Of these, only 30% had early stage tumours. The cases without MNA had significantly higher percentage of early stage tumours than cases with MNA over 10 copies ($P<0.001$) (Table 2).

Treatment and survival rates in patients with MNA

All of the 13 cases with MNA of more than 10 copies received megatherapy with stem cell transplantation and radiotherapy. Six of these cases died. None of 20 cases with MNA from three to nine copies received the megatherapy with stem cell transplantation. Sixteen of the 20 cases received mild chemotherapy, and four cases received only surgical resection without chemotherapy. Only two of the 20 cases died (Table 1). Case 15 had the unresectable tumour of stage III and died of progressive disease although he had received chemotherapy. Case 30 had the resectable tumour with *MYCN* 3 copies by the Southern blot analysis and was not classified into the high-risk group. At 3 months after the operation, this patient had relapse with bone and bone marrow metastasis and died of progressive disease. The primary tumour was judged *MYCN* amplification by the FISH method that was performed after the relapse.

Outcome

Of the 2084 cases that were detected NBs by the screening programme, only 15 cases (0.7%) died within 5 years. OS was 99%. Three-year EFS was 99% for cases without MNA ($n=1500$), 88% for cases with MNA from three to nine copies ($n=20$), and 46% for cases with MNA over 10 copies ($n=13$) ($P<0.001$) (Figure 1). The 4-year OS rate was 99% for cases without MNA, 89% for cases with MNA from three to nine copies and 53% for cases with MNA over 10 copies ($P<0.001$). In the cases with MNA over 10 copies, all of the five cases except one died of progressive disease, though they were received chemotherapy.

Characteristics of patients with and without MNA

Table 2 lists the clinical and biological characteristics of patients with and without MNA. The cases with MNA (>10 copies) were found more frequently in advanced stages (stages III and IV), than the cases without MNA (69 and 23%, respectively; $P<0.001$). Of the cases with MNA (>10 copies), a significantly higher percentage of primary tumours was found in the adrenal glands (92%) than in those without MNA (51%; $P=0.002$). The patients with MNA(>10 copies) had significantly higher serum levels of neuron-specific enolase (NSE) and ferritin than the patients without MNA ($P=0.049$, $P=0.025$, respectively). Although the patients with MNA (>10 copies) had significantly higher urinary levels of HVA than the patients without MNA ($P=0.008$), there was no difference in urinary levels of HVA ($P=0.985$).

Characteristics of patients with MNA

The right side of Table 2 shows clinical and biological characteristics of 33 cases with MNA more than three copies. Patients in advanced stages (stage III and IV) had significantly poorer prognoses (3-year EFS; 58.3%) than those in early stages (stage I, II and IVs) (3-year EFS; 93.3%) ($P=0.021$). The patients with primary tumours found in the adrenal gland had significantly poorer prognoses (3-year EFS; 68%) than those with the tumours at other sites (3-year EFS; 100%) ($P=0.021$). The group with high serum levels of NSE also had a significantly poorer prognosis than the group with low levels of NSE ($P=0.0005$). However, urinary levels of VMA and HVA, and serum levels of ferritin, did not correlate with clinical outcomes ($P=0.364$, 0.478 and 0.174, respectively).

DISCUSSION

It is well known that the prognosis for NB in infants is good. Indeed, the prognosis for NB detected by the Japanese MSPN was excellent, with 98% survival. Although most of the cases detected

Table 1 33 screened patients with MYCN amplification

Case No.	MYCN	Stage	Surgery	Chemotherapy	Radio therapy	Mega therapy	Outcome	Follow-up (year)
1	150	3	CE	VCR, CPM	(−)		NED	0.8
2	>100	4	B	VCR, CPM, VP-16, ADR, CDDP, DTI C	(−)		Tumour death	0.3†
3	55	4	CE	CPM, VP-16, THP-ADR, CDDP, L-PAM, CBDCA	(−)	Auto-BMT	NED	5.3
4	50	4	CE	(+)	(+) 25 gy	PBSCT	Tumour death	2.3†
5	50	4	CE	(+)	(−)		Tumour death	0.7†
6	29	4	CE	CPM, VP-16, THP-ADR, CDDP	(−)	PBSCT	Therapy complication	1.0†
7	24	2	CE	VCR, CPM, VP-16, THP-ADR, CDDP	(−)		NED	5.9
8	20	2	CE	CPM, VP-16, THP-ADR, CDDP	(+) 20 gy	PBSCT	Tumour death	2.7†
9	15	4s	CE	(+)	(−)	Auto-BMT	NED	5.1
10	14	4	B	CPM VP-16, THP-ADR CDDP→refuse	(+) 12 gy		Tumour death	2.5†
11	12	4	CE	(+)	(−)	CBSCT	NED	2.0
12	10	3	CE	VCR, CPM, CDDP, VP-16	(+) 10 gy		NED	3.30
13	10	4s	CE	CPM, VP16, THP-ADR, CDDP	(−)		NED	4.7
14	6	4s	CE	CPM, VP16, THP-ADR, CDDP	(−)		NED	5.0
15	5.7	3	B	VCR, CPM, VP-16, THP-ADR, CDDP	(+) 30 gy		Tumour death	0.9†
16	5	2	CE	VCR, CPM	(+) 24 gy		NED	10.2
17	5	2	CE	VCR, CPM, ADR, CDDP	(−)		NED	8.1
18	4–5	4	CE	VCR, CPM, THP-ADR, CDDP	(−)		NED	8.8
19	4	1	CE	VCR, CPM	(−)		NED	6.6
20	4	1	CE	VCR, CPM	(−)		NED	8.7
21	4	1	CE	VCR, CPM	(−)		NED	6.1
22	4	3	PE	CPM, VP-16, ADR, CDDP	(−)		NED	7.5
23	3.7	4s	CE	VCR, CPM, ADR, CDDP	(−)		NED	6.8
24	3	1	CE	(−)	(−)		NED	5.7
25	3	1	CE	(−)	(−)		NED	5.0
26	3	2	CE	VCR, CPM, THP-ADR, CDDP	(−)		NED	4.5
27	3	2	CE	(−)	(−)		NED	6.0
28	3	3	B	CPM, VP-16, THP-ADR, CDDP	(−)		NED	5.1
29	3	3	CE	VCR, CPM, VP-16, THP-ADR, CDDP	(−)	Auto-BMT	NED	2.1
30	3	3	CE	(−)	(−)		Tumour death	0.9†
31	3	4	CE	CPM, THP-ADR, CDDP	(−)		NED	8.7
32	3	4s	CE	(+)	(−)		NED	7.8
33	2–4	4	B	VCR, CPM, THP-ADR, CDDP	(−)		NED	9.7

by the MSPN had biologically favourable factors, such as no-deletion of 1p and low expression of the *TRK-A* gene, some cases with unfavourable prognostic factors have been reported (Matsunaga *et al*, 2000; Tajiri *et al*, 2001). *MYCN* is one of the most important prognostic factors in NB (Rubie *et al*, 1997; Tonini *et al*, 1997). How *MYCN* is related to the prognosis and clinical features of infantile cases, especially those discovered by MSPN, is not clear. Our large-scale study clarified the frequency and clinical features, including the prognoses, of the infantile NB cases with MNA detected that were detected by MSPN.

Among 1533 cases discovered by the MSPN, 33 cases (2.2%) showed MNA. This frequency is much lower than the 15–22% frequency of MNA cases reported in the United States and Europe (Tonini *et al*, 1997; Brodeur, 2003). In addition, in infants that were less than 1-year-old, the frequency of MNA in our study was lower than that reported in Italy (6.8%) (Tonini *et al*, 1997). This suggests that the MSPN detected a greater number of tumours that spontaneously regressed and/or matured than did the clinical examinations.

MYCN is the powerful prognostic factor in infants whose NB was discovered by the MSPN. The 3-year EFS rates (46%) and 4-year OS rates (53%) for patients with MNA were significantly lower than those for patients without MNA (99.3 and 99%, respectively) ($P<0.001$). According to our previous investigation, the 4-year OS rate for cases less than 12 months old with MNA of over 10 copies, which include clinically detected cases, was 41% (Ikeda *et al*, 2002). The prognosis of cases with MNA detected by MSPN might be comparatively good though prognoses cannot be compared because the researches the survival rates of cases detected clinically and cases detected by MSPN did not investigated at the same time. The infants with MNA that were detected by MSPN might be considered to have benefited from the early detection provided by the screening. Indeed, among patients with MNA, the 3-year EFS rates (93.3%) of patients in stages I, II and IVs were significantly higher than those in stages III and IV (58.3%). If these cases with MNA were not discovered in the early stage by MSPN, some malignant components of tumours would proliferate and progress. As a result, the tumours would be discovered clinically after the patients were 1-year-old. However, the number of cases with MNA is only a very small proportion (2.2%) of the total cases discovered by MSPN. In addition, it is clear that the number of NB patients increased by introduction of MSPN. Therefore, the effectiveness of MSPN discovery of patients with MNA is unclear.

Furthermore, tumours detected by MSPN might regress spontaneously (Yamamoto *et al*, 1998). Several institutions in Japan recently adopted a conservative approach (the 'wait and see' approach), in which children discovered to have stage I, II or IVs tumours by the MSPN were not given any therapeutic treatment in the expectation that the tumour would spontaneously regress (Yamamoto *et al*, 1998). However, a careful follow-up is necessary in cases detected by MSPN, because some of the cases were found to have MNA in the early stage. Most cases with MNA in this study did not have higher urinary VMA levels than without MNA and then, they were not predicted to have a poor clinical outcome at their initial onset. Even in the early stages (stages I, II and IVs), biopsies are required in order to determine the biological prognostic factors of the tumour.

Moreover, in this study, it became clear that patients with MNA of three to nine copies also had poor prognoses. *MYCN* gene has

Table 2 Characteristics of patients with and without *MYCN* amplification detected by mass screening for neuroblastoma

Patient characteristics	Number of cases (%) MNA (>10) ($n=13$)	MNA (3–9) ($n=20$)	MNA (−) ($n=1500$)	*P*-value	MNA (+, >3) 3-yr EFS	*P*-value
Tumour stage						
I	0 (0)	5 (25)	595 (40)	$P<0.001^a$, $P=0.05^b$	100	$P=0.021$
II	2 (15)	5 (25)	463 (31)	(1,2,4s/3,4)	86	(1,2,4s/3,4)
III	2 (15)	5 (25)	280 (19)		71	
IV	7 (54)	2 (10)	65 (4)		44	
IVs	2 (15)	3 (15)	97 (6)		100	
Gender						
Female	3 (23)	11 (55)	722 (49)	$P=0.043^a$, $P=0.579^b$	86	
Male	11 (77)	9 (45)	764 (51)		68	
Primary site						
Adrenal gland	12 (92)	13 (65)	764 (51)	$P=0.002^a$, $P=0.131^b$	68	$P=0.021$
Other abdominal	0 (0)	3 (15)	456 (30)	(adrenal gland/other site)	100	(adrenal gland/other site)
Chest	1 (8)	3 (15)	224 (15)		100	
Pelvis	0 (0)	1 (5)	50 (3)		100	
Neck	0 (0)	0 (0)	6 (0)			
VMA						
$<20\ \mu g\ mgCr^{-1}$	3 (23)	4 (20)	293 (20)		100	
$21-100\ \mu g\ mgCr^{-1}$	7 (54)	15 (75)	982 (67)		82	
$>101\ \mu g\ mgCr^{-1}$	2 (15)	1 (5)	184 (13)		75	
	(mean:			74.6 $\mu g\ mgCr^{-1}$)	(mean: 54.8 $\mu g\ mgCr^{-1}$)	(mean: 54.4 $\mu g\ mgCr^{-1}$)
$P=0.985^a$, $P=0.977^b$		$P=0.364$				
HVA						
$<20\ \mu g\ mgCr^{-1}$	0 (0)	2 (10)	206 (14)		100	
$21-100\ \mu g\ mgCr^{-1}$	7 (54)	16 (80)	1084 (74)		78	
$>101\ ng\ mgCr^{-1}$	6 (46)	2 (10)	170 (12)		63	
	(mean:			107.1 $\mu g\ mgCr^{-1}$)	(mean: 66.0 $\mu g\ mgCr^{-1}$)	(mean: 55.6 $\mu g\ mgCr^{-1}$)
$P=0.008a$, $P=0.371^b$		$P=0.478$				
NSE						
$<15\ ng\ ml^{-1}$	5 (38)	9 (45)	526 (47)		93	
$16-100\ ng\ ml^{-1}$	2 (15)	7 (35)	568 (51)		89	
$>101\ ng\ ml^{-1}$	6 (46)	2 (10)	14 (1)		25	
	(mean: 266.9 $ng\ ml^{-1}$)	(mean: 32.6 $ng\ ml^{-1}$)	(mean: 26.2 $ng\ m^{-1}$l)	$P=0.049^a$, $P=0.285^b$		$P=0.0005$
Ferritin						
$<30\ ng\ ml^{-1}$	2 (15)	5 (25)	506 (54)		100	
$31-100\ ng\ ml^{-1}$	5 (38)	8 (40)	383 (41)		69	
$>101\ ng\ ml^{-1}$	6 (46)	1 (5)	54 (6)		43	
	(mean: 167.3 $ng\ ml^{-1}$)	(mean: 55.9 $ng\ ml^{-1}$)	(mean: 33.7 $ng\ ml^{-1}$)	$P=0.025^a$, $P=0.032^b$		$P=0.174$

[a]*P*-value between MNA (>10) and MNA (−). [b]*P*-value between MNA (3–9) and MNA (−).

been analysed by the Southern blotting method for whole tumours, but this method is not able to evaluate the status MNA in individual NB cells. While, the FISH method is able to evaluate MNA individual tumour cells, however, it is difficult to determine the copy number of MNA by the FISH method. *MYCN* amplification was defined as a more than the fourfold increase of *MYCN* signals in relation to the number of chromosomes 2 in FISH method. Moreover, additional copies up to the fourfold were defined as *MYCN* gain (Spitz *et al*, 2004). Spitz reported that 6% of tumours displayed *MYCN* gain and this *MYCN* gain was associated only with a poor outcome in localized or 4s NB cases (Spitz *et al*, 2004). In our study, these patients with MNA of three to nine copies might suggest the *MYCN* gain rather than *MYCN* amplification. In cases 4 and 30, MNA were confirmed by FISH method, however, in all the cases MNA were not confirmed by it. *MYCN* amplification must be determined by adding the FISH method in these cases (Mathew *et al*, 2001).

In the studies of the USA group (COG) and the German group, the therapeutic strategy of surgical resection or observation is recommended for NB patients in stages I or II, regardless of the presence of MNA (Cohn *et al*, 1995; Kawa *et al*, 1999; Berthold and Hero, 2000; Perez *et al*, 2000). However, in Japan, patients with MNA of more than 10 copies are classified as being in a high-risk group. In the protocol for high-risk NB, patients receive intensive chemotherapy combined with stem cell transplantation (Kawa *et al*, 1999; Kaneko *et al*, 2002). Infantile NB patients with MNA as well as patients in the high-risk group more than 1-year-old with MNA of over 10 copies have been receiving intensive chemotherapy (Matsumura and Michon, 2000). In our study 29 of 33 cases with MNA received chemotherapy regardless of the stage. The use of chemotherapy might improve the prognosis of patients with MNA. In the cases with MNA over 10 copies, the treatment strategy including more intensive chemotherapy might be necessary, because five cases except one died of progressive disease. For cases with

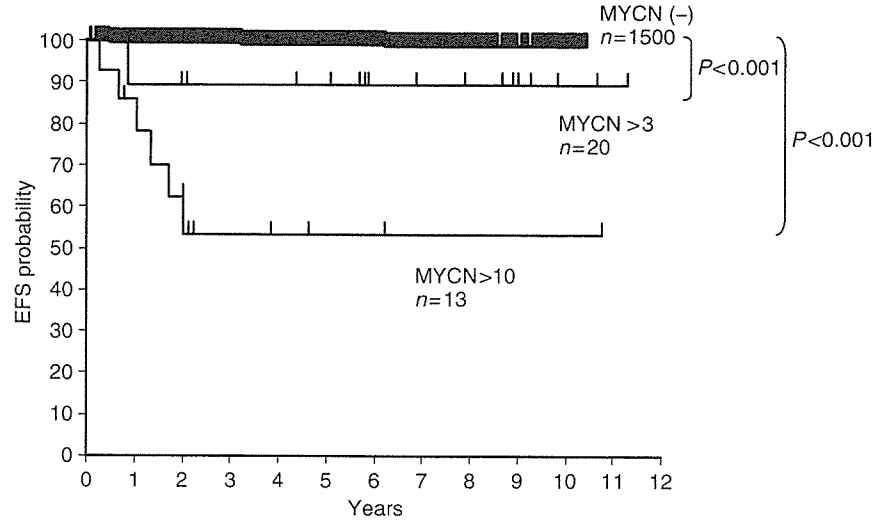

Figure 1 Four-year event-free survival of neuroblastoma infants detected by mass screening based on *MYCN* amplification. The curve was generated with the Kaplan and Meier product limit method. The 4-year OS rate was 99% for patients without MNA, 89% for patients with amplification from three to nine copies, and 53% for patients with more than 10 copies ($P<0.001$).

MNA, it is necessary to establish and perform the appropriate treatment, including not only surgical resection but also chemotherapy.

MYCN amplification was strongly and inversely correlated with the prognosis in infantile cases, although the frequency of MNA in the cases discovered through the MSPN was small (2.2%). Prediction of the presence of MNA in the tumour based on urinary levels of HVA and VMA and stage of the tumour was difficult in the cases we encountered. Our results demonstrate that evaluation of MNA is important for the selection of appropriate treatment for infantile NB.

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Appendix A1

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＜特集「分子標的医療研究の新展開」＞

EGFR 阻害剤（gefitinib/イレッサ®）の小児固形腫瘍における臨床応用への可能性

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The possibility of gefitinib, epidermal growth factor receptor inhibitor, for pediatric solid tumors

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抄　録

小児固形腫瘍の予後は治療の進歩により改善してきた．しかし，難治性の腫瘍に関しては分子標的療法など新規の治療の開発が望まれている．上皮性増殖因子受容体（EGFR）は小児固形腫瘍においても確認されている．また，EGFR チロシンキナーゼ阻害剤のゲフィチニブは腫瘍細胞の増殖を抑制し，非小細胞性肺がんの臨床試験でも有効性が確認されている．有効性の予測因子の結論は出ていないが，EGFR の遺伝子変異，遺伝子の増幅また下流のシグナルの AKT のリン酸化などが報告・議論されている．

我々は，小児固形腫瘍のなかでも特に難治性である悪性横紋筋肉腫様腫瘍（MRT）における EGFR の発現とゲフィチニブの効果を *in vitro* と *in vivo* で検討し，MRT においても *in vitro* と *in vivo* ともに抗腫瘍効果があることを明らかにした．ゲフィチニブは MRT の治療に有効な新規治療薬である可能性を示した．

最近，治療抵抗性の小児固形腫瘍の患児に対するゲフィチニブの第 I 相試験の結果が報告され，その耐用性が示された．ゲフィチニブは小児固形腫瘍の治療において大きな可能性を持つ薬剤であると考えられる．そのためには，腫瘍別に分子標的を明確にし，基礎研究の成果を臨床へと繋ぐ，トランスレーショナルリサーチの基盤整備が必要である．

キーワード：小児固形腫瘍，EGFR，ゲフィチニブ，悪性横紋筋肉腫様腫瘍．

Abstract

The prognoses of pediatric solid tumors (PST) have improved according to progression of therapies. However, current treatments have had only limited success. Then innovative therapies, such as molecular target therapy, are needed. Epidermal growth factor receptor (EGFR) was found to be expressed on some PST. Gefitinib is an oral EGFR-tyrosine kinase inhibitor and has been demonstrated to be effective in inhibiting the proliferation of cancer cells *in vivo* as well as in clinical trials. The effective

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molecular predictors for gefitinib are reported that mutation or gene amplification of EGFR and AKT phosphorylation, however this predictors are controversial.

Among PST, malignant rhabdoid tumor (MRT) is a rare and highly aggressive neoplasm in young children. EGFR was found to be expressed on MRT cell lines and tumor tissues. This encouraged us to examine the antitumor effects of gefitinib on MRT cells *in vitro* and *in vivo*. Gefitinib inhibited EGFR-phosphorylation and *in vitro* cell growth, and a high concentration of gefitinib (20 μM) induced apoptosis *in vitro*. Furthermore, gefitinib had a cytostatic effect on established MRT xenografts. Our results demonstrate that gefitinib has antitumor effects in MRT cells *in vitro* and *in vivo*, and has promise as a novel and therapeutic strategy for MRT.

Recently, the result of Phase I study of gefitinib in children with refractory solid tumor demonstrated that gefitinib is well tolerated. The possibility that gefitinib has the efficacy for PST was demonstrated, however, further clinical studies are needed for the establishment of treatments with gefitinib for PST.

Key words: Pediatric solid tumor, EGFR, Gefitinib, MRT.

はじめに

化学療法，外科治療，放射線治療によるがん治療の進歩により，小児がんの治療成績は目覚しく向上してきた．特に骨髄移植を併用した超大量化学療法や支持療法の進歩は，小児固形腫瘍の予後改善に大きな役割を果たした．しかし，化学療法に用いられてきた従来の抗腫瘍薬は，正常細胞と腫瘍細胞間での選択性が低く，副作用が大きくなるという欠点があった．効果が期待でき，副作用の少ないことが治療薬の理想であり，作用分子の明らかな分子標的薬の開発が進んだ．

がん治療で分子標的薬が注目されるようになった背景には，(1)腫瘍細胞の増殖，浸潤，転移などの悪性化のメカニズムが明らかになり，腫瘍細胞と正常細胞との差異が分子のレベルで明確になったこと，(2)腫瘍細胞周囲の微小環境の研究が進み腫瘍細胞と細胞外器質の関係や転移，浸潤のメカニズムの解明が進んだことがある．これらにより，腫瘍細胞に対する治療の標的が明確になってきた．分子標的療法は，難治性腫瘍に対する，新たな治療法の確立だけではなく，個々の腫瘍の性質，遺伝情報に基づいた個別化した治療（テーラーメイド治療）の可能性を大きくすると期待される．今回，小児悪性固形腫瘍における分子標的療法の現状と今後の可能性について，特にEGFR（epidermal growth factor receptor）チロシンキナーゼ阻害剤であるゲフィチニブ（gefitinib，イレッサ®）を中心に述べる．

EGFRとゲフィチニブ

EGFRは細胞膜を貫通する受容体型チロシンキナーゼで，erbB-1によりコードされる170kDaの糖タンパクである．リガンドとしてEGF, TGF-α, Amphiregulin, β-cellulin, heparin binding-EGF, Epiregulinが知られている．これらのリガンドが受容体に結合すると，二量体を形成し細胞膜内のチロシンキナーゼ部位へのATPの結合が促進し，チロシンキナーゼが活性化する．活性化したチロシンキナーゼからさまざまなシグナル伝達系が活性化され，細胞増殖，生存，浸潤など腫瘍細胞の特性に関与している(図1)．EGFR以外にHER2, HER3, HER4と相同性を有する受容体ファミリーがあることが明らかになり，erbBレセプターファミリーと呼称されるようになり，さらに，erbBレセプターファミリーは同じレセプター同士のhomodimerだけでなく，EGFR/HER2, HER2/HER3といったheterodimerを形成することが知られている[1)2)]．

一方，ゲフィチニブはEGFRチロシンキナーゼ阻害剤として開発された，低分子量の合成アニリノキナゾリンである．チロシンキナーゼが阻害されることで下流のシグナル伝達が阻害され，腫瘍細胞の細胞増殖，生存，浸潤などを抑

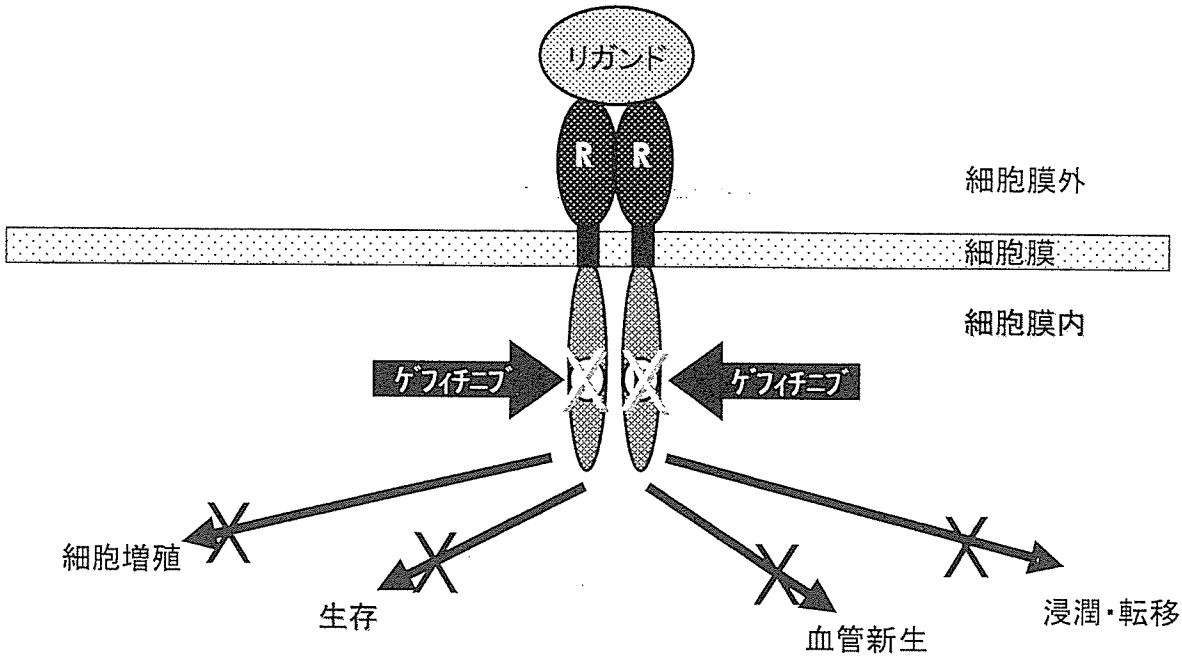

図1　EGFRとゲフィチニブの作用

制し効果を発揮する（図1）．分子標的薬剤では，腫瘍での標的の発現をみれば，治療効果が得られると考えられていた．しかし，ゲフィチニブでは標的となるEGFRの発現量と臨床効果が一致しない結果が得られている[3]．さらに，近年ゲフィチニブの感受性に関して多くの議論がなされている．非小細胞肺がんに発現するEGFRのチロシンキナーゼドメインに変異のある患者での，ゲフィチニブの有効性が[4]報告され，EGFR遺伝子変異が効果の予測に有効である可能性が示された．さらに，2005年にはゲフィチニブの反応性にEGFRの遺伝子のコピー数が重要であること[5]，EGFRの下流に存在するAKTのリン酸化の状態の重要性が指摘されている[6]．また，分子標的治療薬は，がんの原因となった分子を標的にするため，既存の抗がん剤よりも副作用が少ないと考えられていた．しかし，ゲフィチニブでの間質性肺炎など重篤な有害事象の報告から，やはり，リスクとベネフィットを慎重に評価し投与されるべき薬剤である．また，EGFRに変異があっても化学療法とゲフィチニブとの併用療法では生存率に差はなく有効性は証明されなかった[7]．このように，臨床試験が進行していくにつれ，あらたな知見が集積され，次々と問題が提起されているのが現状である．したがって，基礎研究と臨床研究が密接に連携し，分子標的治療の開発を行うことが非常に重要である．

小児固形腫瘍の分子標的と分子標的薬

小児に好発する固形腫瘍である脳腫瘍のなかでもgliomaではEGFRが[8]，神経芽腫にはTrk受容体[9]が，Wilms腫瘍ではHER (human epidermal growth factor receptor)-2[10]が，そしてmedulloblastomaにはHER2とHER4が発現し[11]，予後や悪性化に関与していることが報告されてきた．

したがって，小児固形腫瘍においても，腫瘍細胞の増殖に関与する各種の増殖因子とその受容体で標的分子となりうる．代表的なものとしてTrk, EGFR, HER2, VEGFR (vascular endothelial growth factor receptor), PDGFR (platelet-derived growth factor receptor) あるいはc-Kitなどを挙げることができ，臨床応用できる可能性がある．一方正常細胞には発現していないキメラ遺伝子も標的になる．慢性骨髄性白血病（CML）の*BCR-Abl*遺伝子と急性前骨髄性白血病（APL）のPML/RARαキメラ遺伝子は典型的な分子標的であり，CMLに対してはBCR-