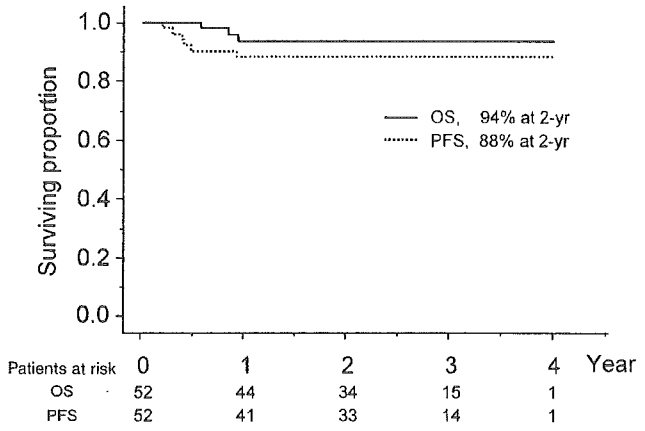

Fig. 1. Overall survival (OS) and progression-free survival (PFS) for eligible patients enrolled in the present study.

and four (9%) were protocol violations. Of the 17 acceptable deviations, one had insufficient margin placement around the primary tumor and 16 had insufficient coverage of regional perigastric lymph nodes expected to receive 30 Gy. All of the four violations consisted of non-compliance in predefined dose constraint to the kidneys. In all cases, the primary tumor was correctly covered in the radiation portals and received 40.5 Gy.

Response and survival. Complete response was achieved in 48 of the 52 patients (92%, 95% confidence interval: 82–98%), non-CR/non-PD in one, and PD in three. Two patients underwent salvage gastrectomy as a result of disease persistence or recurrence after completion of RT. One CR patient developed recurrence in the cerebellum 2 months after the completion of treatment and was alive without disease for 9 months by whole brain RT and salvage chemotherapy. In total, six of the 52 patients experienced disease progression or recurrence. For these six patients, their age ranged 57–65 years; three patients were stage I and three were stage II_1; two patients had elevated LDH before the beginning of the treatment; and five patients had a low-risk IPI and one patient had a low–intermediate risk IPI. We could not find any significant risk factor for treatment failure. Three PD patients died of disease. With a median follow-up period of 28 months, the 2-year progression-free and the 2-year overall survivals were 88 and 94%, respectively (Fig. 1).

Discussion

There has been controversy about the necessity of surgery in the treatment of PGL. Most physicians have recommended gastrectomy as the primary treatment because of good local control and prevention of hemorrhage and perforation. However, resectability in some series can be as low as 66%,[15] and the prognosis of patients with incomplete resection is not good enough.[3,5] Regarding toxicity, in recent reports, the rate of hemorrhage or perforation seemed to be approximately 5% or less after primary chemotherapy with or without RT.[16] These adverse events usually occurred in patients with deep tumoral ulceration and transmural tumor infiltration, and some authors have suggested the use of endoscopic ultrasonography to identify patients fit for surgery.[17,18] In the present study, no gastric hemorrhage or perforation was observed, which might partly have been a result of the patient selection: 48 of the 52 cases had tumor infiltration to the subserosa or less.

A South-west Oncology Group (SWOG) randomized phase III trial showed a favorable 5-year survival of 82% by three cycles of CHOP plus RT compared to that of 72% by eight cycles of CHOP alone in patients with stage I or IE and non-bulky stage II or IIE localized nodal and extranodal aggressive non-Hodgkin's lymphoma.[11] The 5-year overall survival in patients with a low-risk IPI and a low–intermediate risk were 82 and 71%, respectively, including both the CHOP plus RT group and the CHOP alone group. Following these results, we adopted three cycles of CHOP plus RT as a treatment regimen for the present study. The results of the present study showed a favorable 2-year overall survival of 94% in 47 low-risk IPI patients and five low–intermediate IPI risk patients. The German Multicenter Study Group performed a non-randomized trial comparing surgical resection and non-surgical therapy for PGL.[7] All patients in both groups received four cycles of CHOP followed by extended field RT for stage I gastric DLBCL patients or six cycles of CHOP followed by involved field RT for stage II gastric DLBCL patients. The 5-year overall survival was 78% for non-surgical group compared to 79% for surgery group. Although the results of the present study are preliminary with a relatively short follow-up period, the survival figure seems to be similar to the German study also suggesting that gastrectomy is not mandatory, and stomach-preserving treatment should be considered as a reasonable therapeutic option.

Recently updated analysis of the SWOG trial showed an overlapping curve at 9 years for overall survival as a result of late relapses in the CHOP plus RT group, but it remained the standard treatment for stage I and non-bulky stage II patients based on survival advantages through the first 9 years and less toxicity. Late relapses also suggested that optimal treatment may include more or different systemic chemotherapy. Recent reports suggest that rituximab plus CHOP is more effective than CHOP alone in more advanced stage DLBCL[19,20] and the standard treatment for early stage DLBCL patients may be changed in the near future.[21]

One possible disadvantage of RT is long-term toxicities, such as compression fracture of the spine and renal toxicity, which may lead to renal hypertension and renal insufficiency. We observed two cases with compression fracture of the irradiated lower thoracic spine; a possible radiation induced complication. Most of the left kidney also received radiation doses, which could cause renal atrophy, because we allowed traditional antero-posterior and postero-anterior opposed portals. However, Maor *et al.*[22] reported that shrinkage of the irradiated part of the kidney was frequent but clinically significant hypertension or renal dysfunction was not observed if the doses to the kidneys were limited. Today 3-D conformal RT, which makes it easier to reduce doses to normal tissues, is widely used in clinical practice, and it is preferable to traditional RT to reduce possible toxicity in the treatment of PGL.

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References

1. Wotherspoon AC. A critical review of the effect of *Helicobacter pylori* eradication on gastric MALT lymphoma. *Curr Gastroenterol Rep* 2000; **2**: 494–8.
2. Bozzetti F, Audisio RA, Giardini R *et al.* Role of surgery in patients with primary non-Hodgkin's lymphoma of the stomach: an old problem revisited. *Br J Surg* 1993; **80**: 1101–6.
3. Ruskone-Fourmestraux A, Aegerter P, Delmer A *et al.* Primary digestive tract lymphoma: a prospective multicentric study of 91 patients. Groupe d'Etude des Lymphomes Digestifs. *Gastroenterology* 1993; **105**: 1662–71.
4. Bartlett DL, Karpeh MS, Jr. Filippa DA *et al.* Long-term follow-up after curative surgery for early gastric lymphoma. *Ann Surg* 1996; **223**: 53–62.
5. Vaillant JC, Ruskone-Fourmestraux A, Aegerter P *et al.* Management and long-term results of surgery for localized gastric lymphomas. *Am J Surg* 2000; **179**: 216–22.
6. Binn M, Ruskone-Fourmestraux A, Lepage E *et al.* Surgical resection plus chemotherapy versus chemotherapy alone: comparison of two strategies to treat diffuse large B-cell gastric lymphoma. *Ann Oncol* 2003; **14**: 1751–7.
7. Koch P, del Valle F, Berdel WE *et al.* Primary gastrointestinal non-Hodgkin's lymphoma: II. Combined surgical and conservative or conservative management only in localized gastric lymphoma – results of the prospective German Multicenter Study GIT NHL 01/92. *J Clin Oncol* 2001; **15**: 3874–83.
8. Brincker H, D'Amore F. A retrospective analysis of treatment outcome in 106 cases of localized gastric non-Hodgkin lymphomas. Danish Lymphoma Study Group, LYFO. *Leuk Lymphoma* 1995; **18**: 281–8.
9. Raderer M, Valencak J, Osterreicher C *et al.* Chemotherapy for the treatment of patients with primary high grade gastric B-cell lymphoma of modified Ann Arbor Stages IE and IIE. *Cancer* 2000; **88**: 1979–85.
10. Maor MH, Velasquez WS, Fuller LM *et al.* Stomach conservation in stages IE and IIE gastric non-Hodgkin's lymphoma. *J Clin Oncol* 1990; **8**: 266–71.
11. Miller TP, Dahlberg S, Cassady JR *et al.* Chemotherapy alone compared with chemotherapy plus radiotherapy for localized intermediate- and high-grade non-Hodgkin's lymphoma. *N Engl J Med* 1998; **339**: 21–6.
12. Rohatiner A, d'Amore F, Coiffier B *et al.* Report on a workshop convened to discuss the pathological and staging classifications of gastrointestinal tract lymphoma. *Ann Oncol* 1994; **5**: 397–400.
13. Cheson BD, Horning SJ, Coiffier B *et al.* Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol* 1999; **17**: 1244–53.
14. Banno S, Yoshikawa K, Nakamura S *et al.* Monoclonal antibody against PRAD1/cyclin D1 stains nuclei of tumor cells with translocation or amplification at BCL-1 locus. *Jpn J Cancer Res* 1994; **85**: 918–26.
15. Schwarz RJ, Conners JM, Schmidt N. Diagnosis and management of stage IE and stage IIE gastric lymphoma. *Am J Surg* 1993; **165**: 561–5.
16. Maisey N, Norman A, Prior Y *et al.* Chemotherapy for primary gastric lymphoma: does in-patient observation prevent complications? *Clin Oncol* 2004; **16**: 48–52.
17. Fujishima H, Misawa T, Maruoka A *et al.* Staging and follow-up of primary gastric lymphoma by endoscopic ultrasonography. *Am J Gastroenterol* 1991; **86**: 719–24.
18. Palazzo L, Roseau G, Ruskone-Fourmestraux A *et al.* Endoscopic ultrasonography in the local staging of primary gastric lymphoma. *Endoscopy* 1993; **25**: 502–8.
19. Coiffier B, Lepage E, Briere J *et al.* CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346**: 235–42.
20. Pfreundschuh MG, Trumper L, Ma D *et al.* Randomized intergroup trial of first line treatment for patients ≤ 60 years with diffuse large B-cell non-Hodgkin's lymphoma (DLBCL) with a CHOP-like regimen with or without the anti-CD20 antibody rituximab - early stopping after the first interim analysis. *Proc Am Soc Clin Oncol* 2004; **23**: 556a. (Abstract 6500).
21. Wohrer S, Puspok A, Drach J *et al.* Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) for treatment of early-stage gastric diffuse large B-cell lymphoma. *Ann Oncol* 2004; **15**: 1086–90.
22. Maor MH, North LB, Cabanillas FF *et al.* Outcomes of high-dose unilateral kidney irradiation in patients with gastric lymphoma. *Int J Radiat Oncol Biol Phys* 1998; **41**: 647–50.

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SPECIAL ARTICLE

Risks and Benefits of Phase 1 Oncology Trials, 1991 through 2002

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ABSTRACT

BACKGROUND

Previous reviews of phase 1 oncology trials reported a rate of response to treatment of 4 to 6 percent and a toxicity-related death rate of 0.5 percent. These results may not reflect the rates in current phase 1 oncology trials.

METHODS

We reviewed all nonpediatric phase 1 oncology trials sponsored by the Cancer Therapy Evaluation Program at the National Cancer Institute between 1991 and 2002. We report the rates of response to treatment, of stable disease, of grade 4 toxic events, and of treatment-related deaths.

RESULTS

We analyzed 460 trials involving 11,935 participants, all of whom were assessed for toxicity and 10,402 of whom were assessed for a response to therapy. The overall response rate (i.e., for both complete and partial responses) was 10.6 percent, with considerable variation among trials. "Classic" phase 1 trials of single investigational chemotherapeutic agents represented only 20 percent of the trials and had a response rate of 4.4 percent. Studies that included at least one anticancer agent approved by the Food and Drug Administration constituted 46.3 percent of the trials and had a response rate of 17.8. An additional 34.1 percent of participants had stable disease or a less-than-partial response. The overall rate of death due to toxic events was 0.49 percent. Of 3465 participants for whom data on patient-specific grade 4 toxic events were available, 14.3 percent had had at least one episode of grade 4 toxic events.

CONCLUSIONS

Overall response rates among phase 1 oncology trials are higher than previously reported, although they have not changed for classic phase 1 trials, and toxicity-related death rates have remained stable. Rates of response and toxicity vary, however, among the various types of phase 1 oncology trials.

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THE ETHICAL ISSUES RAISED BY PHASE 1 oncology trials have been debated for decades.[1-6] These trials enroll patients with advanced cancer whose disease is usually refractory to available treatment in order to evaluate the safety and toxicity of new therapeutic agents, to establish the pharmacokinetic properties of those agents, and to determine a safe dose for subsequent testing.[7] Published reviews report that a tumor response occurs in 4 to 6 percent of the participants in these trials and that about 0.5 percent of participants die as the result of toxicity.[8-16] Critics of such trials cite these data when raising concerns about the poor prospect of benefit and the potential for severe harm. Some contend that the enrollment of patients with advanced disease in risky research studies with little chance of direct benefit exploits a vulnerable population.[17] The response rates of 4 to 6 percent and the toxicity-related death rate of 0.5 percent continue to be viewed as representative of phase 1 oncology trials, but these rates are based on reviews of single-agent trials. They do not take into full account the development of new types of anticancer agents, trials of combinations of agents, new trial designs, or improvements in supportive care, and they do not present a comprehensive picture of the benefits and risks associated with phase 1 trials.[18-20]

To better inform the discussion of the risks and benefits involved in phase 1 oncology trials, we reviewed studies that began between 1991 and 2002 and were sponsored by the Cancer Therapy Evaluation Program of the National Cancer Institute, the major sponsor of phase 1 oncology trials in the United States. Reflecting the full spectrum of phase 1 oncology trials, our review included trials of chemotherapeutic agents and newer, targeted agents such as antiangiogenesis factors, vaccines, and gene therapies; trials of combinations of agents, including some already approved by the Food and Drug Administration (FDA); and published and unpublished trials. To extend our understanding of the benefits and risks associated with phase 1 oncology research, data on stable disease and grade 4 toxic events are reported in addition to conventional measures of outcome.

METHODS

All nonpediatric phase 1 oncology trials sponsored by the Cancer Therapy Evaluation Program that began between 1991 and 2002 were eligible for this review, including trials that evaluated solid tumors and hematologic cancers and trials conducted at the National Institutes of Health (NIH) Clinical Center and other institutions around the United States. Excluded were phase 1–phase 2 trials, trials of radiation therapy alone, of stem-cell or bone marrow transplantation, of supportive care without anticancer agents, and of therapies for diseases other than cancer (e.g., human immunodeficiency virus disease).

The staff of the Cancer Therapy Evaluation Program plan, review, coordinate, and oversee clinical trials of investigational anticancer agents.[21] The program receives comprehensive trial data at regular intervals from investigators and actively monitors all trials through routine data submission and periodic audits. Between 1991 and 2002, data from phase 1 trials sponsored by the Cancer Therapy Evaluation Program were monitored by five different sources: the Clinical Trials Monitoring System, the Clinical Data Update System, the Annual Update System, the Quarterly Data Update, and Study Summary reports.

The Clinical Trials Monitoring System, which has been managed for the Cancer Therapy Evaluation Program by Theradex since 1979, is a database of electronically submitted case-report forms for first trials of agents in humans as well as trials of combinations of investigational new drugs and at least one FDA-approved drug that may be associated with a risk of overlapping toxic effects. Extensive data are submitted every two weeks for quality control and are maintained in a relational KnowledgeMan database (Micro Data Base Systems). Each participating institution is audited for quality assurance three times a year.

The Clinical Data Update System, managed by Capital Technology Information Systems, has received electronic data according to course of therapy and according to patient every three months since 1998. The Clinical Data Update System is generally used for late phase 1 trials of agents whose toxicity profile has been established in earlier studies. Data are maintained in a relational Oracle database. Before 1998, summary data for these trials were submitted as paper reports yearly (by the Annual Update System or by Study Summary reports), quarterly (by Quarterly Data Update), or twice a year in printed trial summaries prepared by the cooperative groups. For trials monitored by the Clinical Data Update System, the Annual Update System, Study Summary reports, and Quarterly Data Update,

each institution is audited every three years. Auditors examine the consistency of reporting, including references to source documents concerning toxic events among subjects and assessments of responses. Data reported in this article include selected variables from the database of the Cancer Therapy Evaluation Program and combine data from the program's five monitoring sources. A subgroup of 110 trials, primarily those monitored by the Annual Update System, was excluded because complete data in regard to toxicity were unavailable. None of the excluded trials were from the Clinical Trials Monitoring System's database of studies involving agents used for the first time in humans, studies involving agents filed as investigational new drugs with the FDA, or other early phase studies. The Cancer Therapy Evaluation Program provided the data on May 16, 2003.

Trials were grouped by an experienced investigator of phase 1 trials into one of six categories according to the mechanism of action of the agent or agents under investigation: cytotoxic chemotherapeutic agents, immunomodulators, receptor-transduction or signal-transduction agents (including those affecting gene reexpression), antiangiogenesis agents, gene-transfer agents, and vaccines. Each of these categories was further subdivided into four types of trials: those for single investigational agents, for multiple investigational agents, for both investigational and FDA-approved agents, and for only those agents approved by the FDA. Trials involving multiple investigational agents with different mechanisms of action were grouped according to the agent predicted to be the most toxic. Thus, any trial involving a combination of therapies that included a chemotherapeutic investigational agent was coded as a chemotherapy trial, and any trial that included an immunomodulating investigational agent but no chemotherapeutic agents was categorized as an immunomodulator trial. Trials that included both investigational and FDA-approved agents were categorized according to the mechanism of action of the investigational agent. For purposes of classification, radiation was considered an FDA-approved agent.

In cases in which the study title identified a specific disease, the study was considered disease-specific. Studies of single investigational cytotoxic chemotherapeutic agents were labeled "classic" phase 1 trials. Studies of agents being used in humans for the first time were selected from all five databases. These included the very first study of an agent conducted after the agent was filed as an investigational new drug with the FDA and trials that were initiated within seven months of the first study, before any information was available about dose-limiting toxicity from the very first trial.

Potentially beneficial effects of agents under investigation were categorized as complete response, partial response, less-than-partial response, and stable disease. Response to treatment was reported for each protocol according to guidelines of the World Health Organization (WHO),[22] the Response Evaluation Criteria in Solid Tumors,[23] or other established criteria approved by the Protocol Review Committee of the Cancer Therapy Evaluation Program. A complete response was defined as the disappearance of a tumor; a partial response as an overall 50 percent reduction in the tumor, measured as the sum of the products of the two longest diameters (according to the WHO criteria), or as an overall 30 percent reduction in tumor size, measured as the sum of the longest diameters (according to guidelines of the Response Criteria in Solid Tumors); and stable disease as neither a partial response nor progressive disease.[23] For this analysis, less-than-partial response and stable disease are combined into one category.

Toxicity was reported with the use of the Common Toxicity Criteria.[24] Protocols specified which version of these criteria were used, depending on when the protocols were initiated. All deaths reported by investigators as "possibly," "probably," or "definitely" related to treatment were considered toxicity-related deaths. Data on patient-specific grade 4 toxic events that were available from the Clinical Data Update System are reported; for the other trials, only the data on cumulative toxicity according to trial were available.

STATISTICAL ANALYSIS

Response rates, death rates, and rates of grade 4 toxic events were calculated for participants who were assessed according to trial category (i.e., therapeutic modality, single agent or combination, disease-specific or not, and first-in-human or other). Rates were calculated by dividing the total number of events (responses, deaths, or grade 4 toxic events) by the total number of patients assessed for response or toxicity. Response rates and toxicity-related death rates were also calculated for three-year intervals to evaluate trends. For the subgroup of trials monitored by the Clinical Data Update System, the percentage of patients who had grade 4

toxic events and the average number of grade 4 toxic events per affected patient were reported. Comparisons of response rates and of toxicity-related death rates — in particular, between the current sample and prior samples — were made descriptively. Calculation of statistical significance was intentionally avoided in cases where patient samples may have been divergent and hypothesis testing not prospectively defined. Statistical analyses were performed with the use of SAS software, version 8.02.

RESULTS

The sample of 460 phase 1 oncology trials sponsored by the Cancer Therapy Evaluation Program

Table 1. **Rates of Response to Treatment in Phase 1 Oncology Trials.**

Trial	No. of Trials	No. of Patients Assessed for Response	Rate of Response			
			Overall Response (Complete and Partial)	Complete Response	Partial Response	Stable Disease and Less-Than-Partial Response
			percent			
Total	460	10,402	10.6	3.1	7.5	34.1*
Cytotoxic chemotherapy						
One investigational agent	92	2,341	4.4	1.5	2.9	40.8
Multiple investigational agents	12	273	11.7	1.5	10.3	27.5
Combination of investigational and FDA-approved agents	88	2,251	16.4	5.6	10.8	31.3†
FDA-approved agents only	29	792	27.4	8.0	19.4	27.2†
Immunomodulator						
One investigational agent	13	203	11.3	3.0	8.4	35.5
Multiple investigational agents	28	651	6.9	2.2	4.8	22.3†
Combination of investigational and FDA-approved agents	19	392	26.0	5.6	20.4	26.7†
Receptor or signal transduction						
One investigational agent	51	1,347	3.2	0.7	2.5	39.3
Multiple investigational agents	7	81	7.4	1.2	6.2	27.2
Combination of investigational and FDA-approved agents	61	935	11.7	2.1	9.5	37.4
Antiangiogenesis						
One investigational agent	15	335	3.9	0.6	3.3	31.0
Combination of investigational and FDA-approved agents	9	135	14.8	5.2	9.6	37.0
Gene transfer						
One investigational agent	7	89	3.4	0	3.4	30.3
Combination of investigational and FDA-approved agents	1	3	0	0	0	0
Vaccine						
One investigational agent	15	265	3.4	3.0	0.4	24.9
Multiple investigational agents	7	198	1.0	1.0	0	35.4
Combination of investigational and FDA-approved agents	6	111	5.4	2.7	2.7	19.8

* For 630 of 10,402 participants, data on stable disease and less-than-partial response are not reported. The percentage was calculated with 9772 as the denominator.

† Percentages were calculated with a denominator adjusted to exclude participants for whom data on stable disease and less-than-partial response were unavailable.

that opened between 1991 and 2002 included 11,935 participants. All participants were assessed for toxicity, and 10,402 were assessed for a response (Table 1). Trials of cytotoxic chemotherapeutic agents accounted for 48.0 percent (221) of all trials and for 54.4 percent (5657) of participants assessed for response. Trials involving receptor transduction or signal transduction were the second-largest group (119 trials, or 25.9 percent), representing 22.7 percent (2363) of participants assessed for response. There were only eight trials involving gene transfer, with 92 participants (Table 1).

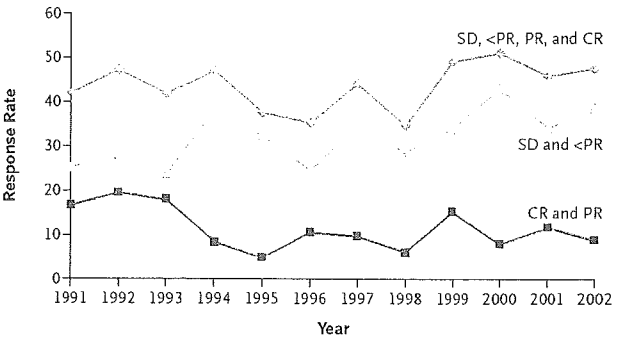

Figure 1. **Response Rates According to Year.**

Response to therapy was classified as complete (CR), partial (PR), less than partial (<PR), or as stable disease (SD). When the rates were grouped according to three-year periods, a downward trend was observed for complete and partial responses, but when stable disease and less-than-partial responses were taken into account, the rate remained relatively constant over time.

RESPONSE RATES

Among the trials of all types of agents, 10.6 percent of the 10,402 participants assessed for response had either a partial or a complete response to therapy. Of these, 7.5 percent had a partial response and 3.1 percent had a complete response. In addition, 34.1 percent of the participants in phase 1 trials had either stable disease or a less-than-partial response (Table 1).

Response rates varied according to the type of agent used and the characteristics of the trial (Table 1). The overall response rate was 3.0 percent among trials of vaccines and 13.6 percent among studies of immunomodulators (data not shown). Furthermore, response rates varied within categories according to the type of trial. For classic phase 1, single-agent chemotherapy studies, the overall response rate was 4.4 percent. The rate among chemotherapy studies involving more than one investigational agent was 11.7 percent; for combinations of investigational and FDA-approved agents, the rate was 16.4 percent; and for phase 1 trials including only FDA-approved chemotherapeutic agents, the rate was 27.4 percent (Table 1). A similar variation was seen in the other categories of trials (Table 1). The response rate among 3420 participants in 184 disease-specific trials was 19.3 percent; among trials that were not specific to disease, the rate was 6.3 percent.

Response rates also varied over time, with the highest rate (19.5 percent) occurring in 1992 and the lowest (5.0 percent) in 1995. When the rates were grouped according to three-year periods, a downward trend in complete and partial responses was noted (18.3 percent for 1991 to 1993 and 9.4 percent for 2000 to 2002). However, when stable disease was taken into account, the rate remained relatively constant over time (34.6 to 51.3 percent) (Fig. 1).

TOXICITY

Among the 11,935 participants in all 460 phase 1 studies, there were 58 deaths (0.49 percent) that were determined to be at least possibly related to the treatment (Table 2). Of those deaths, 18 were reported as definitely related to the treatment and 7 as probably related (for a combined toxicity-related death rate of 0.21 percent). When calculated in three-year intervals for 1991 through 2002, the toxicity-related death rate remained relatively constant (range, 0.45 to 0.61 percent). Of the 58 deaths, 43 (74.1 percent) occurred in participants in chemotherapy trials, with the highest toxicity-related death rate (0.77 percent) occurring in trials involving both investigational and FDA-approved agents (Table 2). Classic phase 1 trials of single investigational chemotherapeutic agents had a toxicity-related death rate of 0.57 percent. Thirteen deaths were reported among trials of receptor-transduction or signal-transduction agents (0.47 percent) and one death each among trials of immunomodulators (0.07 percent) and antiangiogenesis factors (0.17 percent). There were no reported deaths in phase 1 gene-transfer or vaccine studies.

In a subgroup of 168 studies that involved 3465 patients assessed for toxicity, 14.3 percent of participants had had grade 4 toxic events; an average of 1.9 grade 4 events occurred per affected patient (Table 3). On average, trials of chemotherapeutic agents were associated with the highest rate of tox-

Table 2. Deaths from Toxic Events in Phase 1 Oncology Trials.

Trial	No. of Trials	No. of Patients Assessed for Toxic Events	Deaths from Toxic Events*
			no. (%)
Total	460	11,935	58 (0.49)
Cytotoxic chemotherapy			
One investigational agent	92	2,621	15 (0.57)
Multiple investigational agents	12	305	2 (0.66)
Combination of investigational and FDA-approved agents	88	2,594	20 (0.77)
FDA-approved agents only	29	925	6 (0.65)
Immunomodulator			
One investigational agent	13	235	0
Multiple investigational agents	28	730	1 (0.14)
Combination of investigational and FDA-approved agents	19	443	0
Receptor or signal transduction			
One investigational agent	51	1,565	3 (0.19)
Multiple investigational agents	7	99	2 (2.02)
Combination of investigational and FDA-approved agents	61	1,081	8 (0.74)
Antiangiogenesis			
One investigational agent	15	402	0
Combination of investigational and FDA-approved agents	9	171	1 (0.58)
Gene transfer			
One investigational agent	7	107	0
Combination of investigational and FDA-approved agents	1	5	0
Vaccine			
One investigational agent	15	297	0
Multiple investigational agents	7	218	0
Combination of investigational and FDA-approved agents	6	137	0

* Deaths include all those reported as possibly, probably, or definitely related to the treatment.

icity, with 17.4 percent of participants experiencing at least one grade 4 toxic event; vaccine trials had the lowest rate, with no grade 4 toxic events reported (Table 3). Among all 11,935 participants assessed in the 460 studies, 5251 grade 4 toxic events were reported.

FIRST-IN-HUMAN TRIALS

Of 460 trials, 117 (25.4 percent) involving a total of 3164 participants assessed for a response to therapy were considered first-in-human trials — that is, studies designed to establish initial information on toxicity and dose for agents not previously tested in humans (Table 4). The overall response rate in these studies was 4.8 percent, as compared with 13.1 percent in the other studies. The toxicity-related death rate in first-in-human studies was 0.26 percent, as compared with 0.58 percent in studies not considered first-in-human trials. Studies of cytotoxic chemotherapeutic agents made up the largest group of first-in-human trials (36.8 percent). Of the vaccine studies sponsored by the Cancer Therapy Evaluation Program, 82.1 percent were first-in-human trials.

Table 3. Grade 4 Toxic Events in Phase 1 Oncology Trials.

Trial	No. of Trials	No. of Patients Assessed for Toxic Events	Patients with a Grade 4 Toxic Event	Average No. of Grade 4 Toxic Events per Patient
			%	
Total	168	3465	14.3	1.9
Cytotoxic chemotherapy				
One investigational agent	20	408	15.0	1.6
Multiple investigational agents	3	23	4.3	2.0
Combination of investigational and FDA-approved agents	17	475	14.5	1.8
FDA-approved agents only	3	159	34.0	2.4
Immunomodulator				
One investigational agent	2	43	2.3	1.0
Multiple investigational agents	10	207	9.7	2.2
Combination of investigational and FDA-approved agents	5	101	4.0	1.8
Receptor or signal transduction				
One investigational agent	29	839	13.0	1.7
Multiple investigational agents	6	67	19.4	2.0
Combination of investigational and FDA-approved agents	51	752	18.1	2.0
Antiangiogenesis				
One investigational agent	9	143	5.6	1.6
Combination of investigational and FDA-approved agents	6	101	17.8	1.8
Gene transfer				
One investigational agent	1	26	11.5	1.7
Combination of investigational and FDA-approved agents	1	5	0	0
Vaccine				
One investigational agent	3	20	0	0
Multiple investigational agents	2	96	0	0

TRIALS WITH FDA-APPROVED AGENTS

Overall, 213 studies (46.3 percent) included at least one FDA-approved anticancer agent. Response rates were higher in trials with FDA-approved agents than in trials without FDA-approved agents (Table 5). These studies had an overall response rate of 17.8 percent, as compared with 4.8 percent for studies not including FDA-approved anticancer agents. The toxicity-related death rate was higher (0.65 percent) than for trials that did not include FDA-approved anticancer agents (0.35 percent).

DISCUSSION

We comprehensively reviewed phase 1 oncology trials sponsored by the Cancer Therapy Evaluation Program between 1991 and 2002. The overall response rate in these trials was 10.6 percent, which is higher than previously reported, whereas the toxicity-related death rate, 0.49 percent, is similar to that of previous reports. Rates of response and of toxicity-related death among classic phase 1 trials of single chemotherapeutic agents are similar to those reported in other reviews, but classic trials account for only 22 percent of participants in this review.

Response rates in phase 1 oncology trials have been reported to be 4 to 6 percent, with toxicity-related death rates reported to be 0.5 percent or lower.[8-16] In our review, however, we found that response rates in recent phase 1 oncology trials exceeded 10 percent, with stable disease or less-than-partial re-

Table 4. Response Rates and Deaths from Toxic Events in Phase 1 Oncology Trials Involving the First Use of an Agent in Humans.

Trial	No. of Trials	No. of Patients Assessed for Response	Overall Response Rate*	No. of Patients Assessed for Toxic Events	Deaths from Toxic Events†
			%		*no. (%)*
Total					
First use of an agent in humans	117	3164	4.8	3498	9 (0.26)
All other trials	343	7238	13.1	8437	49 (0.58)
Cytotoxic chemotherapy					
First use of an agent in humans	43	1298	5.0	1422	7 (0.49)
All other trials	178	4359	15.0	5023	36 (0.72)
Immunomodulator					
First use of an agent in humans	16	404	7.4	431	1 (0.23)
All other trials	44	842	16.6	977	0
Receptor or signal transduction					
First use of an agent in humans	27	742	3.8	853	1 (0.12)
All other trials	92	1621	8.0	1892	12 (0.63)
Antiangiogenesis					
First use of an agent in humans	8	200	7.0	228	0
All other trials	16	270	7.0	345	1 (0.29)
Gene transfer					
First use of an agent in humans	0	0	0	0	0
All other trials	8	92	3.3	112	0
Vaccine					
First use of an agent in humans	23	520	3.1	564	0
All other trials	5	54	1.9	88	0

* The overall response rate includes both complete and partial responses.
† Deaths include all those reported as possibly, probably, or definitely related to the treatment.

sponse having been achieved in an additional 34.1 percent of participants. Rates of toxicity-related death have not increased over time, and more than 85 percent of participants had no grade 4 toxic events. As compared with other reviews, these data suggest that participants may benefit more from current phase 1 oncology trials than previously believed.

A recent review of single-agent trials showed that there was a decrease in tumor-response rates over time,[13] which was attributed to the use of newer, more specific agents and changes in trial design. In our review, response rates per year varied without a clear pattern. When these rates were grouped in three-year intervals, there was a decrease in complete or partial responses from 1991 to 2002 but an increase in rates of stable disease. Little change in the benefit to participants over time was seen when response rates were grouped with stable disease.

In our view, it is inaccurate to refer to phase 1 oncology studies as if they are all similar to one another. Nearly half of the trials we studied included at least one FDA-approved agent, and less than half included chemotherapeutic agents. Different types of phase 1 oncology studies are associated with very different response rates. For instance, the response rate among patients who were treated with immunomodulators was 13.6 percent, yet the rate was just 3.0 percent for patients treated with vaccines. Trials that included one or more FDA-approved anticancer agents showed higher response rates than did those involving only investigational agents. For these reasons, it may be misleading to summarize phase 1 oncology trials with the use of a single response rate.

Risk, as measured by toxicity-related death rates and grade 4 toxic events, also varies according to the type of trial. The average toxicity-related death rate for trials of cytotoxic chemotherapeutic agents was 0.67 percent but just 0.07 percent for those in-

Table 5. Response Rates and Deaths from Toxic Events in Phase 1 Oncology Trials, According to Whether FDA-Approved Agents Were Used.

Trial	No. of Trials	No. of Patients Assessed for Response	Overall Response Rate*	No. of Patients Assessed for Toxic Events	Deaths from Toxic Events†
			%		*no. (%)*
Single investigational agent	193	4580	4.2	5227	18 (0.34)
Multiple investigational agents	54	1203	7.1	1352	5 (0.37)
Combination of investigational and FDA-approved agents	184	3827	15.8	4431	29 (0.65)
FDA-approved agents only	29	792	27.4	925	6 (0.65)

* The overall response rate includes both complete and partial responses.
† Deaths include all those reported as possibly, probably, or definitely related to the treatment.

volving immunomodulators, and no toxicity-related deaths were reported in gene-transfer or vaccine trials. Grade 4 toxic events were more common in chemotherapy trials, especially those involving multiple agents, than in all other trials. Trials of FDA-approved drugs, which evaluated the safety of higher doses or combinations of drugs, appeared to be associated with the highest rates of toxicity (a death rate from toxic events of 0.65 percent, vs. 0.35 percent for other trials) but also had the highest overall response rate (17.8 percent, vs. 4.8 percent for other trials). Overall, newer, nonchemotherapeutic agents are associated with lower rates of toxic events.

Classic phase 1 studies of single investigational chemotherapeutic agents, which were the only trials included in previous reviews, showed an overall response rate of 4.4 percent and a toxicity-related death rate of 0.57 percent. These rates are almost identical to those previously reported.[8-16] In this study of trials sponsored by the Cancer Therapy Evaluation Program and initiated between 1991 and 2002, classic phase 1 trials accounted for only 22 percent of all participants. Similarly, the testing of investigational agents never before studied in humans is commonly thought of as a defining characteristic of phase 1 oncology trials. In our review, these first-in-human studies represented less than a quarter of phase 1 studies and enrolled less than a third of participants. Response rates, but also toxicity-related death rates, are lower in studies that test agents for the first time in humans than in those that do not test agents for the first time.

When the risks and benefits associated with phase 1 oncology trials are weighed, factors other than response rates and toxicity should be taken into account. Investigational treatments may have clinically meaningful benefits — reduced pain, increased appetite, energy, and activity, weight gain, reduced fatigue, or increased ability to perform daily activities.[20,25,26] Some of these benefits might accrue from research participation itself; for some persons, contributing to research and potentially helping future cancer patients may also be an important benefit.[27] At the same time, participation in research may involve additional burdens: multiple visits or long hours at the clinic, unpleasant procedures, and the possible financial costs associated with participation in research studies.[28]

This study has several limitations. First, our data are derived only from trials sponsored by the Cancer Therapy Evaluation Program. Although the program is a major sponsor of phase 1 oncology trials in the United States[29] and the use of data from the program avoids publication bias, any differences that might be found in the phase 1 trials with other sponsors have not been captured. It is possible that the response rates associated with trials of promising agents sponsored by pharmaceutical companies could be higher than those reported here. Second, for trials involving gene transfer, the findings should be interpreted with caution because of the small number of trials and the possibility that outliers influenced the data. Finally, our reporting of grade 4 toxic events is limited. Patient-specific data on grade 4 toxic events came from one monitoring source, which, although it includes some first-in-human trials, is generally used to monitor later phase 1 studies and may not be entirely representative of phase 1 oncology studies. Moreover, the data on grade 4 toxic events are reported without distinguishing among the types of toxic events.

Since not all toxic events have similar medical consequences, evaluation of the risks in phase 1 trials should include both the types and the frequency of events experienced by participants.

In conclusion, reliance on a single estimate of the response rate or the toxicity-related death rate for phase 1 oncology trials is misleading, since rates of response and toxicity vary according to the type of trial. Potential participants and their families, oncologists, investigators, members of institutional review boards, ethicists, and others interested in weighing the risks and benefits of phase 1 studies and making decisions about their acceptability should be aware of the complexity and variety of such trials, know the details about the trial they are considering, and carefully evaluate all relevant risks and benefits.

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REFERENCES

1. Lipsett MB. On the nature and ethics of phase I clinical trials of cancer chemotherapies. JAMA 1982;248:941-2.
2. Kodish E, Stocking C, Ratain MJ, Kohrman A, Siegler M. Ethical issues in phase I oncology research: a comparison of investigators and institutional review board chairpersons. J Clin Oncol 1992;10:1810-6.
3. Agrawal M, Emanuel EJ. Ethics of phase 1 oncology studies: reexamining the arguments and data. JAMA 2003;290:1075-82.
4. Miller M. Phase I cancer trials: a collusion of misunderstanding. Hastings Cent Rep 2000;30:34-43.
5. *Idem*. Phase I cancer trials: a crucible of competing priorities. Int Anesthesiol Clin 2001;39:13-33.
6. Emanuel EJ. A phase I trial on the ethics of phase I trials. J Clin Oncol 1995;13:1049-51.
7. Markman M. The needs of science vs the needs of patients: ethical concerns in cancer clinical trials. Cleve Clin J Med 2003;70:1008-9, 1013-4, 1016.
8. Itoh K, Sasaki Y, Miyata Y, et al. Therapeutic response and potential pitfalls in phase I clinical trials of anticancer agents conducted in Japan. Cancer Chemother Pharmacol 1994;34:451-4.
9. Estey E, Hoth D, Simon R, Marsoni S, Leyland-Jones B, Wittes R. Therapeutic response in phase I trials of antineoplastic agents. Cancer Treat Rep 1986;70:1105-15.
10. Decoster G, Stein G, Holdener EE. Responses and toxic deaths in phase I clinical trials. Ann Oncol 1990;1:175-81.
11. Bachelot T, Ray-Coquard I, Catimel G, et al. Multivariable analysis of prognostic factors for toxicity and survival for patients enrolled in phase I clinical trials. Ann Oncol 2000;11:151-6.
12. Han C, Braybrooke JP, Deplanque G, et al. Comparison of prognostic factors in patients in phase I trials of cytotoxic drugs vs new noncytotoxic agents. Br J Cancer 2003;89:1166-71.
13. Roberts TG Jr, Goulart BH, Squitieri L, et al. Trends in the risks and benefits to patients with cancer participating in phase 1 clinical trials. JAMA 2004;292:2130-40.
14. Sekine I, Yamamoto N, Kunitoh H, et al. Relationship between objective responses in phase I trials and potential efficacy of nonspecific cytotoxic investigational new drugs. Ann Oncol 2002;13:1300-6.
15. Smith TL, Lee JJ, Kantarjian HM, Legha SS, Raber MN. Design and results of phase I cancer clinical trials: three-year experience at M.D. Anderson Cancer Center. J Clin Oncol 1996;14:287-95.
16. Von Hoff DD, Turner J. Response rates, duration of response, and dose response effects in phase I studies of antineoplastics. Invest New Drugs 1991;9:115-22.
17. Annas GJ. The changing landscape of human experimentation: Nuremberg, Helsinki, and beyond. Health Matrix Clevel 1992;2:119-40.
18. Dent SF, Eisenhauer EA. Phase I trial design: are new methodologies being put into practice? Ann Oncol 1996;7:561-6.
19. Eisenhauer EA, O'Dwyer PJ, Christian M, Humphrey JS. Phase I clinical trial design in cancer drug development. J Clin Oncol 2000;18:684-92.
20. Chen EX, Tannock IF. Risks and benefits of phase 1 clinical trials evaluating new anticancer agents: a case for more innovation. JAMA 2004;292:2150-1.
21. Ansher SS, Scharf R. The Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute: industry collaborations in new agent development. Ann N Y Acad Sci 2001;949:333-40.
22. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47:207-14.
23. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205-16.
24. Cancer Therapy Evaluation Program. Common Toxicity Criteria (CTC), version 2.0. Bethesda, Md.: National Cancer Institute, 1999. (Accessed February 14, 2005, at http://ctep.cancer.gov/reporting/ctc.html.)
25. Markman M. Partial responses to antineoplastic drug therapy: what do they mean? J Cancer Res Clin Oncol 1992;119:5-6.
26. Shanafelt TD, Loprinzi C, Marks R, Novotny P, Sloan J. Are chemotherapy response rates related to treatment-induced survival prolongations in patients with advanced cancer? J Clin Oncol 2004;22:1966-74.
27. Daugherty CK, Siegler M, Ratain MJ, Zimmer G. Learning from our patients: one participant's impact on clinical trial research and informed consent. Ann Intern Med 1997;126:892-7.
28. Goldman DP, Berry SH, McCabe MS, et al. Incremental treatment costs in National Cancer Institute-sponsored clinical trials. JAMA 2003;289:2970-7.
29. Takimoto CH. Anticancer drug development at the US National Cancer Institute. Cancer Chemother Pharmacol 2003;52:Suppl 1:S29-S33.

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Special Report

Learning from a Visit to the JNCI Editorial Office

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INTRODUCTION

We, the Managing Editor and Statistical Advisor of the *Japanese Journal of Clinical Oncology* (JJCO), had a chance to visit the *Journal of the National Cancer Institute* (JNCI) editorial office in Bethesda, Maryland, in the USA. As is generally known, JNCI is acclaimed as the source for the most up-to-date information in the field of cancer research. It is also the most-cited academic journal in oncology. Whilst there we took the opportunity to interview the Executive Editor, Dr Rebecca Chasan, and the Managing Editor, Mr Mark Leader (Fig. 1). They graciously spent more than 3 hours with us. As the information that they shared with us is highly valuable for both editorial staff and authors, we have summarized the major points from the interview and draw comparisons between JNCI and JJCO.

OVERVIEW

JNCI is published by Oxford University Press (OUP). It is not affiliated with the United States National Cancer Institute (NCI), although originally JNCI was founded at NCI as a government publication. OUP took over ownership in 1997 mainly to develop an online version of the journal. JNCI accepts papers that give insight into mechanisms and processes involved in cancer prevention, development, screening, and treatment.

REVIEW PROCESS

Figure 2(a) and (b) indicate the review process for initial submission of manuscripts for JNCI and JJCO. At both journals, the Editor-in-Chief rejects some manuscripts on initial submission, the remainder are forwarded to Associate Editors. The Associate Editors reject some manuscripts and send others to reviewers for peer review. Those which are peer-reviewed

Figure 1. JNCI editorial office. From left to right: K. Hashimoto (Managing Editor JJCO), R. Chasan (Executive Editor JNCI), M. Leader (Managing Editor JNCI).

can be rejected or provisionally accepted, depending on the outcome of the peer review process. A notable difference between JNCI and JJCO is the existence of Senior Editors. Senior Editors do not exist at JJCO. The JNCI senior editors, all of whom have PhD degrees, are involved in the entire review process to help the Editor-in-Chief of JNCI, but their main function is to edit manuscripts to ensure that the presentation is as clear and logical as possible. They are concerned not only with scientific issues but also with the wording and presentation of the manuscript. As the journal's target audience is relatively broad, it is not necessary that the Senior Editors be medical doctors. Most have a background in molecular biology.

ONLINE JOURNAL AND ONLINE SUBMISSION/REVIEW SYSTEM

JJCO is published in electronic form on the Internet. Full-text articles can be accessed through HighWire Press

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(a) Review Process in JNCI

Author → EA → SE →EIC → AE → SE → EA → Reviewer → SE → SR
→ SE → AE →SE → EIC, SE → EIC → Author

EA: Editorial Assistant, SE: Senior Editor, EIC: Editor-in-Chief, AE: Associate Editor
SR: Statistical Reviewer

(b) Review Process in JJCO

Author → ME → EIC, EE → AE → Reviewer → AE → EIC, EE → Author

ME: Managing Editor, EIC: Editor-in-Chief, EE: Executive Editor, AE: Associate Editor

Figure 2. (a) Review process in JNCI. (b) Review process in JJCO.

(http://jjco.oupjournals.org/). The online version of JNCI, JNCI Cancer Spectrum (http://jncicancerspectrum.oupjournals.org/), contains not only the full content of the printed journal but also weekly updated news and useful cancer information including cancer statistics, drug information, and NCI's Physician Data Query (PDQ).

Recently, the number of medical journals that employ an online submission and peer review system has increased substantially. The online system reduces the time and correspondence expense required in the reviewing process. At JNCI an online submission and peer review system was launched in January 2004. Since the start of online submission, the number of submissions has increased by approximately 20%. The transition from conventional paper submission to online submission was very smooth, and JNCI now receives very few conventional submissions. At JJCO the online submission system started in 2003. The number of submissions has dramatically increased by 50% since the online system launched.

COMMENTS FROM THE MANAGING EDITOR OF JJCO

I have been Managing Editor of JJCO for 3 years, which is my first experience as a journal editor. I have learned how to be a journal editor through trial and error and with the help of other more experienced staff; however, I have not had many opportunities to discuss editorial processes and difficulties. An interview with the editorial staff of a first-class scientific journal was a tremendous opportunity for me to assess the editorial process of JJCO. JNCI receives a much larger number of submissions than JJCO and the review process is much more complicated. Nonetheless, the editorial review process at JNCI runs smoothly without any noticeable bottlenecks. Overall, I found, through the interview, that the approach I took in managing JJCO was appropriate. For example, as is the case with JJCO, the editorial staff of JNCI emphasize the importance of receiving comments from assigned reviewers in a timely manner.

As mentioned above, the number of manuscripts submitted to JJCO has increased strikingly since online submission started, while the number of editorial staff has remained the same. The sudden growth in submissions has led to an increase in the volume of work that the editorial office has had to handle at various stages of the review process. One of the important tasks in the review process is the selection of reviewers. At JJCO, Associate Editors generally choose two reviewers from among 160 Editorial Board Members for each manuscript. This sharp rise in submissions has imposed a heavy burden on the Associate Editors and Editorial Board Members of JJCO. Therefore, the number of reviewers needs to be increased to reduce the burden. Also, a wide variety of experts' comments are important for the fair evaluation of manuscripts. JNCI has adopted an external reviewer system for peer review, and as they have a huge database of external reviewers, they are able to obtain a broad range of opinion. At JJCO we have decided to increase greatly the number of reviewers in order to improve the reviewing process. We expect to solve the workload problem to some extent in this way; however, a number of issues still remain to be considered.

The JNCI staff introduced us to a useful organization, the Council of Science Editors (CSE) (http://www.councilscienceeditors.org/index.cfm) to help find ways to alleviate our problems and to improve our skills. Members of CSE include editors of many prestigious scientific journals such as JNCI, *Lancet*, *Nature*, *Journal of the American Medical Association*, and *Journal of Clinical Oncology*. CSE improves communication in science by educating authors and editors. Also, CSE promotes effective communication practices in publishing. On a personal level I have to engage in a special effort to improve my skills as an editor through communication and experience.

COMMENTS FROM THE STATISTICAL ADVISOR OF JJCO

Editorial work on a scientific journal needs a very broad knowledge base. During our visit to the JNCI editorial office I asked the Executive Editor about the difficulties associated with dealing with broad areas of science. She stressed the importance of the participation of multidisciplinary reviewers and added that the requirement of a very broad knowledge base, by the Editor-in-Chief, about cancer research is important. This is refelected by the fact that the Editor-in-Chief, and a JNCI, Dr Barnett S. Kramer, is also the Editor-in-Chief, and a member of several PDQ editorial boards. One reason that the

editorial office of JNCI works very effectively seems to be related to their system, which is established so as to directly implement the Editor-in-Chief's ideas.

Another point I was curious about is the role of the Senior Editor. Senior Editors play a very important role in the JNCI system. A similar function is used at *Science* and at the *New England Journal of Medicine*, though it is not very common at other journals, including JJCO. As noted above, the Senior Editor serves as a bridge between authors, reviewers, and other editors. The Senior Editor's role is not only administrative, but also scientific.

I'd like to introduce my experience with the Senior Editor when our paper was published in the JNCI (J Natl Cancer Inst 2003;95:906–13). For example, my original manuscript included the following statement:

> Among the contents of soybeans, isoflavones, a group of phytoestrogens has been hypothesized to have a protective effect against the development of breast cancer.

The Senior Editor changed this as follows:

> Soybeans contain isoflavones, a group of phytoestrogens, that have been hypothesized to have reduce the risk of breast cancer.

The reason for the wording change was explained as the journal avoids use of the phrase "protective effect" unless the results are derived from a randomized, controlled trial.

In another example the original sentence said:

> Other possible mechanisms enabling soybean isoflavones to be anticarcinogenic are inhibition of protein tyrosine kinases and other enzyme activities, stimulations of sex hormone binding globulin production, antioxidant effects, and inhibition of angiogenesis.

The change by the Senior Editor included some questions to authors as follows:

> Other possible anticarcinogenic mechanisms associated with soy or isoflavones include inhibiting protein tyrosine kinases and other enzyme activities that **[Au: do what? interfere with cell growth and survival?]**, stimulating sex hormone-binding globulin production that **[Au: does what?]**, antioxidant effects protecting DNA from damage **[Au: correct?]**, and inhibiting angiogenesis.

Figure 3. JNCI editorial office. From left to right: S. Yamamoto (Statistical Advisor JJCO) and J. Watson (Senior Editor JNCI).

As shown in these examples, the role of the Senior Editor is to make sure that the manuscript is readable by the general audience. Her suggestions were generally very reasonable and improved the quality of my manuscript. Of course, some points she made were not correct in a strict scientific sense, but we were able to communicate with each other via e-mail and reached agreement without a significant time lag, which was surprising since we have a 13-hour time difference. I was very glad to meet the Senior Editor, Dr Joanna Watson (Fig. 3), who dealt with my manuscript, when I visited the JNCI editorial office.

The quality of JNCI is kept high by this efficient system and by the continuous effort of editorial staff. The visit was very fruitful in that we got to personally experience the system employed on this quality journal. It also confirmed to us that the system used on JJCO is appropriate. It is the intention of the editorial staff of JJCO to provide a high level of service to both readers and authors through the continued publication of a quality journal involving collaborative efforts with readers, authors, and the editorial staff of other journals.

CpG Island Methylator Phenotype Is a Strong Determinant of Poor Prognosis in Neuroblastomas

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Abstract

Neuroblastoma, one of the most common pediatric solid tumors, is characterized by two extreme disease courses, spontaneous regression and life-threatening progression. Here, we conducted a genome-wide search for differences in DNA methylation that distinguish between neuroblastomas of the two types. Three CpG islands (CGI) and two groups of CGIs were found to be methylated specifically in neuroblastomas with a poor prognosis. By quantitative analysis of 140 independent cases, methylation of all the five CGI (groups) was shown to be closely associated with each other, conforming to the CpG island methylator phenotype (CIMP) concept. The presence of CIMP was sensitively detected by methylation of the *PCDHB* CGIs and associated with significantly poor survival (hazard ratio, 22.1; 95% confidence interval, 5.3-93.4; $P < 0.0001$). Almost all cases with N-*myc* amplification (37 of 38 cases) exhibited CIMP. Even in 102 cases without N-*myc* amplification, the presence of CIMP (30 cases) strongly predicted poor survival (hazard ratio, 12.4; 95% confidence interval, 2.6-58.9; $P = 0.002$). Methylation of *PCDHB* CGIs, located in their gene bodies, did not suppress gene expression or induce histone modifications. However, CIMP was significantly associated with methylation of promoter CGIs of the *RASSF1A* and *BLU* tumor suppressor genes. The results showed that neuroblastomas with CIMP have a poor prognosis and suggested induction of silencing of important genes as an underlying mechanism. (Cancer Res 2005; 65(3): 828-34)

Introduction

Epigenetic abnormalities, especially alterations in DNA methylation, are intimately involved in development of various human tumors (1). Aberrant methylation of promoter CpG islands (CGI) causes inactivation of tumor suppressor genes. Genomic instability is caused by genomic hypomethylation and is associated with hypermethylation (2, 3). Identification of epigenetic abnormalities in human cancers is expected to lead not only to discovery of novel disease mechanisms but also to development of new diagnostic markers. Therefore, we previously developed a genome-wide scanning method, methylation-sensitive representational difference analysis (MS-RDA), for detecting differences in DNA methylation (4, 5). This technique analyzes unmethylated, CpG-rich regions of the genome and has already identified genes silenced in human lung, stomach, breast, and pancreatic cancers (6–9).

Neuroblastoma derived from primitive cells of the sympathetic nervous system is one of the most common solid tumors in childhood, characterized by two extreme disease courses, spontaneous regression, and life-threatening progression (10, 11). The clinical outcome is associated with disease stage, age at diagnosis, histologic classification, N-*myc* amplification, DNA ploidy, and *TrkA* overexpression (10–12). These characteristics are therefore used to classify cases into low-, intermediate-, and high-risk groups. However, especially in the cases with intermediate risk, prediction of prognosis and therapeutic decision-making are still difficult, and development of new markers is an urgent priority. Moreover, the molecular bases underlying the two distinct clinical courses are still unknown, and their clarification is needed to allow development of novel therapeutics.

In the present study, considering the major involvement of epigenetic machinery in embryonic development (13, 14), we searched for differences in DNA methylation between neuroblastomas with a good prognosis and counterparts with a poor prognosis by MS-RDA.

Materials and Methods

Tissue Samples and Cell Lines. Tumor samples were obtained from 145 nonrecurrent cases between 1995 and 1999 and were used under approval of institutional review boards. The mean age at initial diagnosis was 27 months (range, 0-216 months). Their clinical stages were determined according to the International Neuroblastoma Staging System, and 40, 17, 20, 60, and 8 cases belonged to stages I, II, III, IV, and IVS, respectively. Normal adrenal medulla tissue was collected from a case undergoing nephrectomy for a renal cancer. Neuroblastoma cell lines were obtained from the American Type Culture Collection (Manassas, VA), the Japanese Collection of Research Bioresources (Tokyo, Japan), and the RIKEN Bio Resource Center (Tsukuba, Japan). GANB was established by A.N. and normal human bronchial epithelial cells were purchased from Cambrex (East Rutherford, NJ). High molecular weight DNA and total RNA were extracted as previously described (7). Total RNAs of brain and adrenal glands were purchased from Clontech (Palo Alto, CA).

MS-RDA and Database Search. MS-RDA was done as previously described (4, 5). Genomic DNA of primary neuroblastomas with a good prognosis (cases 92, 98, 104, 112, and 148) and neuroblastoma cell lines established from cases with a poor prognosis (CHP134, IMR32, GANB, NGP, and TGW) were digested with *Hpa*II, and then two pooled DNA samples were prepared. Although use of cell lines is highly recommended for MS-RDA (5), no cell lines were available for neuroblastomas with a good prognosis, and therefore we used the primary samples. To isolate CGIs that were hypermethylated in the latter, the cell line pool was used as the tester, and the primary tumor pool as the driver. MS-RDA in the opposite direction

Note: Supplementary data for this article are available at Cancer Research online (http://cancerres.aacrjournals.org/).

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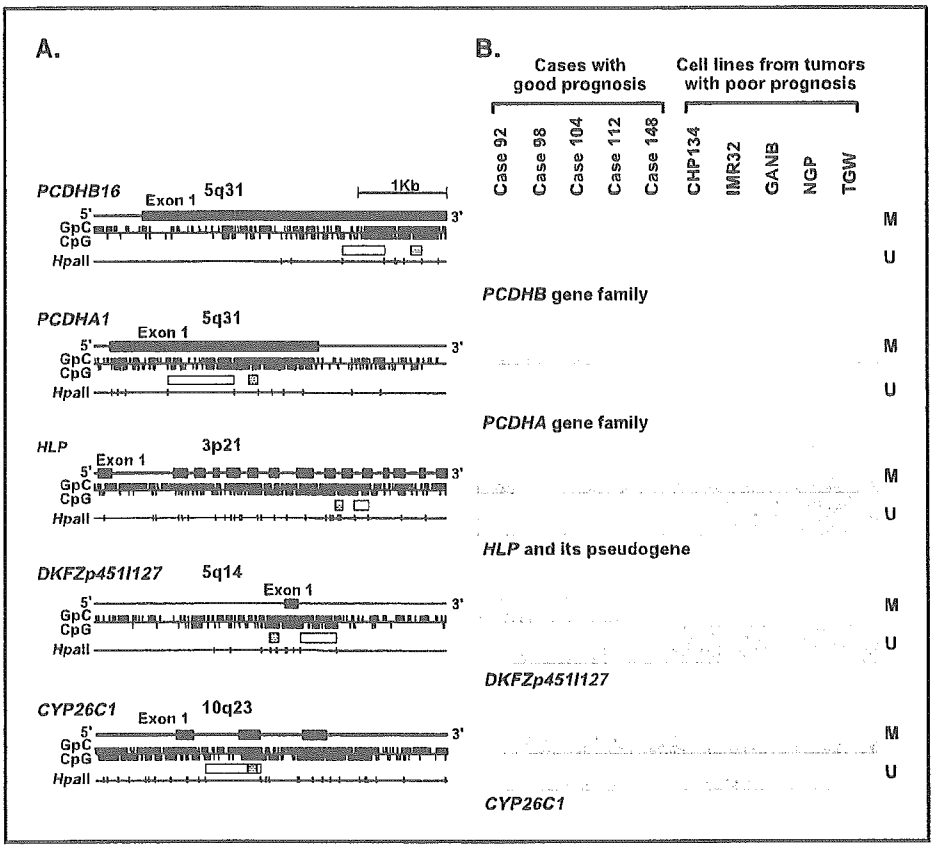

Figure 1. Five CGIs isolated by MS-RDA and their methylation statuses in the samples used for MS-RDA. *A*, genomic structures of the five CGIs. GpC, CpG, and *Hpa*II recognition sites (5′-CCGG-3′) are shown by ticks. *Closed boxes*, exons; *open boxes*, clones isolated by MS-RDA; *shaded boxes*, regions analyzed by MSP. *B*, methylation statuses analyzed by MSP. *M*, MSP using primers specific to methylated DNA; *U*, MSP using primers specific to unmethylated DNA. All the five CGIs were found to be differentially methylated between the two groups used for MS-RDA.

was also done. For each series of MS-RDA, 96 clones were analyzed for redundancy, and nonredundant clones were sequenced. Their genomic origins were examined using BLASTN software http://www.ncbi.nlm.nih.gov/BLAST/).

Sodium Bisulfite Modification and Methylation-Specific PCR. One microgram of DNA underwent sodtlbium bisulfite modification (15), and was suspended in 20 μL of TE buffer. For methylation-specific PCR (MSP), 1 μL of the solution was used for PCR with primers specific to methylated or unmethylated sequences. Using DNA from normal human bronchial epithelial and DNA methylated with *Sss*I methylase, annealing temperatures specific for methylated and unmethylated primers were determined. Quantitative MSP was done separately for methylated DNA molecules and for unmethylated DNA molecules. Standard DNA was prepared by cloning PCR products amplified by methylated and unmethylated primers into a vector, respectively. The numbers of methylated and unmethylated molecules in a test sample were determined by comparing their amplification with those of standard samples containing 10 to 10^6 molecules. The "methylation index" was calculated as the fraction of methylated molecules in the total DNA molecules (no. methylated molecules + no. unmethylated molecules). Each sample was analyzed twice, blind to clinical information, and high reproducibility was confirmed (correlation coefficient = 0.98).

The *protocadherin* β (*PCDHB*) family consists of 16 genes with single exons and three pseudogenes on 5q31, and their CGIs are located in the gene bodies. MSP primers were designed to recognize 17 of the 19 members (all except for the *PCDHB1* gene and the *PCDHB19* pseudogene). The *protocadherin* α (*PCDHA*) family consists of 15 genes and one pseudogene having unique first exons and shared exons 2 to 4 on 5q31, and their CGIs are located in exon 1. MSP primers were designed to recognize 13 of the 16 members (all except for the *PCDHAC1* and *PCDHAC2* genes and the *PCDHA14* pseudogene). The *hepatocyte growth factor-like protein* (*HLP/MSP/MST1*) gene is highly homologous to *macrophage stimulating, pseudogene 9* (*MSTP9*), and MSP primers were designed to recognize both of these. For *DKFZp451I127*, *FLJ37440*, *Zinc finger protein 297* (*ZNF297*), and *Cytochrome p450 CYP26C1* (*CYP26C1*), MSP primers were designed to recognize each of them specifically. The primers and PCR conditions are shown in Supplementary Table 1.

Semiquantitative and Quantitative Reverse Transcription-PCR. cDNA was synthesized from 3 μg of total RNA treated with DNase using a Superscript II kit (Invitrogen Co., Carlsbad, CA). For semiquantitative reverse transcription-PCR (*PCDHB1-PCDHB15*), multiple cycles of PCR were tested for each gene, and numbers giving a wide dynamic range were determined. The primers and PCR conditions are shown in Supplementary Table 2. For quantitative reverse transcription-PCR (*PCDHB16*), the number of cDNA molecules was determined by quantitative PCR, as in quantitative MSP, and the copy number was normalized to that of *GAPDH*.

Chromatin Immunoprecipitation Assay. From 1×10^6 cells, DNA/histone complexes were immunoprecipitated, and DNA was eluted in 30 μL of TE after reversing cross-linking. Copy numbers of DNA molecules of the *PCDHB16* exon, *RASSF1A* promoter, and *GAPDH* promoter in 1 L of the eluate were determined by quantitative PCR (primer sequences in Supplementary Table 3), and normalized to the copy numbers in the input. Anti-acetyl-histone H3 antibody (AcH3) and anti-dimethylated-histone H3 (lysine 9; MetH3K9) were purchased from Cell Signalling (Beverly, MA).

Statistical Analysis. Associations between methylation levels among CGI groups were examined using the Pearson correlation coefficient and Fisher's exact test. Survival time was measured from the date of initial diagnosis to the date of death or last contact. Kaplan-Meier analysis and log-rank tests were done to compare survival between the groups defined by methylation levels. Hazard ratio (HR) between groups and dose-response relationships between methylation levels and survival were estimated by the Cox proportional hazard model. Kaplan-Meier curves were drawn with the help of Aabel software (Gigawiz. Ltd. Co., Tulsa, OK) and other analyses were conducted using SAS version 8.2 (SAS Institute, Inc., Cary, NC).

Results

Genome-Scanning for Differentially Methylated CpG Islands. MS-RDA was done using five primary neuroblastomas with a good prognosis and five neuroblastoma cell lines established from cases with a poor prognosis. Seven DNA fragments, derived from CGIs of *PCDHB16*, *PCDHA1*, *HLP*, *DKFZp451I127*, *FLJ37440*, *ZNF297*, and *CYP26C1*, were isolated as methylated in the latter samples. No DNA fragments were isolated as methylated in the former samples. Methylation statuses of (i) 17 CGIs of the *PCDHB* family (detailed structure in Supplementary Fig. 1), (ii) 13 CGIs of the *PCDHA* family, (iii) *HLP* and its pseudogene, and (iv) other four unique CGIs were examined by MSP. This revealed that the *PCDHB* family (5q31), the *PCDHA* family (5q31), *HLP* (3p21) and its pseudogene (1p36), *DKFZp451I127* (5q14), and *CYP26C1* (10q23) were specifically methylated in the latter samples (Fig. 1*A* and *B*).

Close Association between Methylation and Poor Prognosis in 140 Independent Primary Samples. To analyze the significance of the differential methylation of the above five CGI (groups) in primary neuroblastomas, 140 primary samples, all different from the initial five samples, were analyzed by quantitative MSP. When distributions of methylation indices were analyzed (Fig. 2), a clear bimodal distribution was observed for (i) the CGI group in the *PCDHB* family (17 CGIs), (ii) the CGIs of *HLP* and its pseudogene, and (iii) the *CYP26C1* CGI. The results thus indicated that the cases could be classified into two groups, one with high methylation and the other with low methylation. The dose-response relationships between high *PCDHB* methylation and poor prognosis were analyzed by the Cox proportional model using the methylation index as a continuous value, and the association was confirmed with a trend $P < 0.0001$. Normal adrenal medulla had a methylation index of 4%.

According to the bimodal distribution, the effect of high methylation was assessed by dichotomous groups. For the *PCDHB* family, cutoff values of 30%, 40%, 50%, 60%, 70%, and 80% were tested, and HRs of 16.8 [95% confidence interval (95% CI), 4.0-70.9], 22.1 (95% CI, 5.3-93.4; Fig. 3), 13.1 (95% CI, 4.5-37.9), 9.1 (95% CI, 3.8-23.4), 7.0 (95% CI, 3.1-15.8), and 7.8 (95% CI, 3.4-17.6), respectively, were obtained ($P < 0.001$ for all cutoff values). This showed that cases can be classified into two groups with distinct prognoses, and we adopted a cutoff value of 40%, which gave the highest HR, for convenience in the following analysis.

The dose-response relationships were also confirmed for other four CGI (groups), *PCDHA* ($P = 0.004$), *HLP* ($P < 0.0001$), *DKFZp451I127* ($P = 0.02$), and *CYP26C1* ($P < 0.0001$). Cutoff values were similarly tested, and those for *PCDHA*, *HLP*, *DKFZp451I127*, and *CYP26C1* were set at 80%, 10%, 20%, and 70%, respectively, with HRs of 5.7 (95%CI, 1.4-24.0; $P = 0.07$), 21.7 (95% CI, 5.1-91.4; $P < 0.0001$), 3.2 (95% CI, 1.0-10.5; $P = 0.045$), and 8.7 (95% CI, 4.1-18.1; $P < 0.0001$), respectively (Fig. 3).

Existence of the CpG Island Methylator Phenotype in Neuroblastomas. Methylation of the different CGI (groups) had shown close associations with each other (Table 1). When correlation was analyzed as a continuous value, Pearson correlation coefficients between *PCDHB* and *PCDHA*, *HLP*, *DKFZp451I127* and *CYP26C1* were 0.55, 0.70, 0.26 and 0.77, respectively. This showed that multiple CGIs were simultaneously methylated in

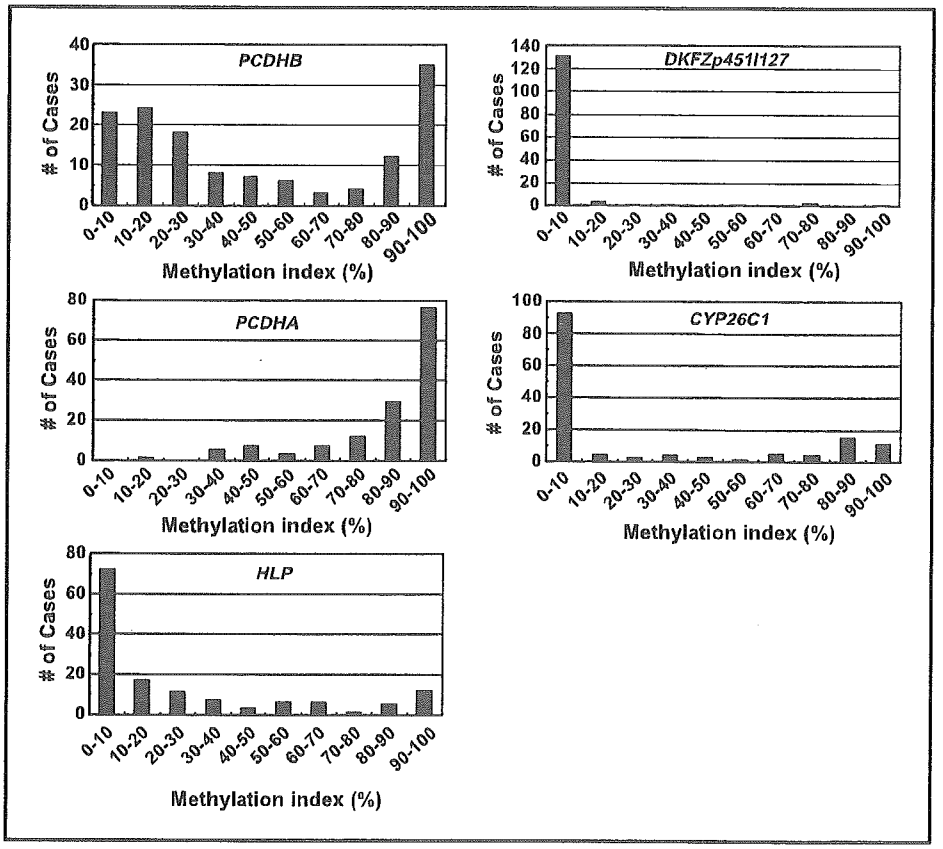

Figure 2. The distribution of methylation indices among the 140 cases analyzed: (*i*) 17 CGIs of the *PCDHB* family, (*ii*) 13 CGIs of the *PCDHA* family, (*iii*) CGIs of *HLP* and its pseudogene, (*iv*) *DKFZp451I127*, and (*v*) *CYP26C1*.

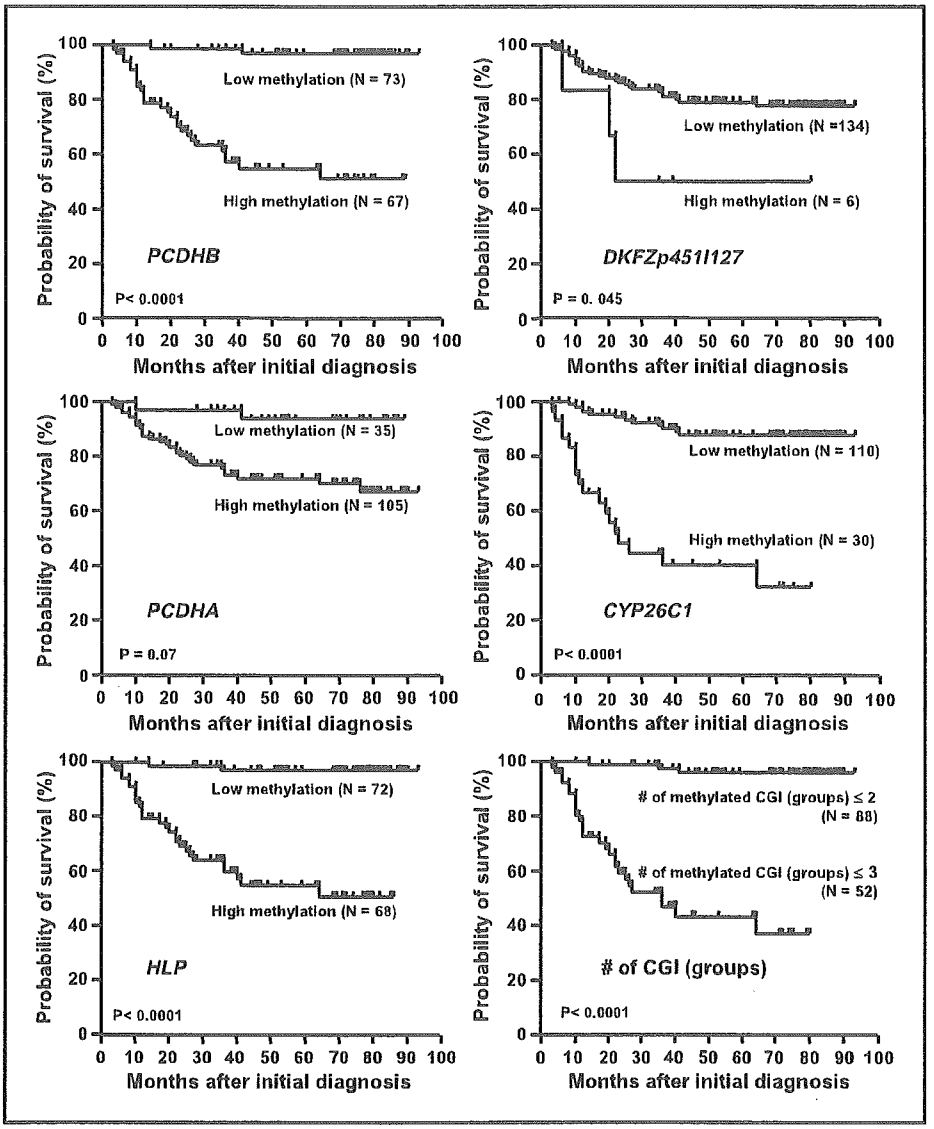

Figure 3. Predictive powers of methylation of the five CGI (groups) identified, and their multiple methylation: (*i*) 17 CGIs of the *PCDHB* family, (*ii*) 13 CGIs of the *PCDHA* family, (*iii*) CGIs of *HLP* and its pseudogene, (*iv*) *DKFZp451I127*, (*v*) *CYP26C1*, and (*vi*) methylation of three of these or more were analyzed by the Kaplan-Meier method using 140 primary samples. The *PCDHB* family, *HLP*, *DKFZp451I127*, *CYP26C1*, and methylation of multiple CGI (groups) had significant influence on survival.

neuroblastomas with a poor prognosis (Supplementary Fig. 2*A*). The simultaneous methylation of (i) 17 CGIs of the *PCDHB* family, (ii) 13 CGIs of the *PCDHA* family, (iii) CGIs of *HLP* and its pseudogene, (iv) *DKFZp451I127* CGI, and (v) *CYP26C1* CGI conformed with the concept of the CpG island methylator phenotype (CIMP; ref. 16).

Associations between CIMP and poor prognosis were examined by defining CIMP as cases with methylation of two CGI (groups) or more, those with three or more, those with four or five, and those with five. When CIMP was defined as cases with methylation of three CGI (groups) or more, the largest association with poor prognosis was observed, with a HR of 25.4 (95% CI, 7.6-84.5; Fig. 3). However, the HR (22.1) given by 17 CGIs of the *PCDHB* gene family approximated to this, and the *PCDHB* methylation level closely correlated with the number of methylated CGI (groups; Supplementary Fig. 2*B*). Therefore, for simplicity of analysis, we defined CIMP in neuroblastomas on the basis of high methylation of the *PCDHB* family, tentatively with a cutoff value of 40%.

Predictive Power of CIMP, Compared with Known Prognostic Factors. Univariate analyses showed that N-*myc* amplification, low *TrkA* expression, DNA diploidy, and an age no younger than 1 year gave HRs of 9.5 (95% CI, 4.4-20.5), 3.9 (95% CI, 1.7-9.3), 4.2 (95% CI, 1.65-10.8), and 12.3 (95% CI, 3.7-41.7). Cases were stratified by these known factors (Table 2). In those without N-*myc* amplification, CIMP also showed an influence with a HR of 12.4 (95% CI, 2.6-58.9), but almost all cases with N-*myc* amplification (37 of the 38 cases) showed CIMP. It was suggested that cases with N-*myc* amplification were contained in the cases with CIMP. CIMP was independent from *TrkA* overexpression, DNA ploidy, and age at diagnosis. Stage seemed to be a stronger prognostic factor. Notably, even when limited to cases in stages III and IV without N-*myc* amplification, which are classified into the intermediate risk group and clinically important, CIMP gave a HR of 4.8 (95% CI, 1.0-23.0; $P = 0.048$).

Multivariate analyses were finally done taking all the five known prognostic factors into account. Although CIMP gave a HR of 5.0 (95% CI, 0.47-52.7), it was not significant ($P = 0.18$), possibly due to limitation in the number of cases.

Table 1. Association between the *PCDHB* methylation and methylation of other CGIs

Variables	Methylation level of *PCDHB* family gene		*P**
	High (≥40%)	Low (<40%)	
No. cases (n = 140)	67	73	
Methylation of CGIs outside promoter regions (n = 140)			
PCDHA gene family (exon 1)†	65/67	41/73	<0.0001
HLP (exons 2-13)‡	52/67	16/73	<0.0001
CYP26C1 (exon 2)§	30/67	0/73	<0.0001
p41Arc (intron 8)	1/67	1/73	0.48
SIM2 (exon 2)	0/67	0/73	
Methylation of CGIs in promoter regions (n = 140)			
DKFZp451I127‖	6/67	0/73	0.011
RASSF1A	51/67	10/73	<0.0001
BLU	25/67	3/73	<0.0001
p16	0/67	0/73	
hMLH1	0/67	0/73	
PCDHB1	0/67	0/73	
TAF7	0/67	0/73	
p41Arc	0/67	0/73	
SIM2	0/67	0/73	

*Fisher's exact test.
†Boundaries for high methylation and low methylation of *PCDHA* gene family were set at 80% of the methylation index.
‡Boundaries for high methylation and low methylation of *HLP* were set at 10% of the methylation index.
§Boundaries for high methylation and low methylation of *CYP26C1* were set at 70% of the methylation index.
‖Boundaries for high methylation and low methylation of *DKFZp451I127* were set at 20% of the methylation index.

Effects of PCDHB Methylation on Gene Expression and Chromatin Structure. The CGIs of the *PCDHB* family were located in their gene bodies, whose methylation generally does not block gene transcription (17). The actual effects of methylation on expression were examined for 16 genes of the *PCDHB* family using 10 primary neuroblastomas with low methylation and five primary neuroblastomas with high methylation. The methylation was not associated with loss of expression (a representative result is shown in Fig. 4*A*). The effect of methylation of the *PCDHB16* CGI on the histone modification was further examined by chromatin immunoprecipitation assay. It was found that DNA methylation of the *PCDHB16* CGI did not induce histone H3 lysine 9 methylation or histone H3 deacetylation (data not shown).

Association between CIMP and Promoter Methylation. High methylation of *PCDHB* CGIs, a sensitive surrogate marker of CIMP in neuroblastomas, did not repress gene expression or induce histone modification. This indicated that CIMP is involved in the poor prognosis of neuroblastomas by causing methylation of promoter CGIs, although it is known that promoter CGIs are resistant to *de novo* methylation (18, 19).

Among the five CGI (groups) identified in this study, only that of *DKFZp451I127* was located in a promoter region. Although its methylation was infrequent, the methylation was observed only in neuroblastomas with CIMP (Table 1), and was associated with expression loss (Fig. 4*B*). To make the association clearer, methylation statuses were analyzed for eight additional CGIs in promoter regions. It was shown that methylation of promoter CGIs of *RASSF1A* (3p21) and *BLU* (3p21) was far more frequently observed in neuroblastomas with CIMP (Table 1, $P < 0.0001$). At the same time, there was a preference for CGIs affected by CIMP among CGIs in promoter regions, and also among those outside promoter regions (Table 2).

Discussion

Extensive methylation of multiple CGIs, conforming with the concept of CIMP, was here found specifically present in neuroblastomas with a poor prognosis and could be sensitively detected by focusing on the *PCDHB* family. *PCDHB* methylation did not suppress gene expression or induce histone modification. However, CIMP was associated with promoter methylation of *RASSF1A* and *BLU* genes and one of the mechanisms underlying the poor prognosis of neuroblastomas seemed to be silencing of these and possibly other tumor suppressor genes and genes important for differentiation.

CIMP was originally identified in colon cancers (16), but there has been some dispute over its presence (20). The clear correlation between CIMP and a poor prognosis found here for neuroblastomas was unequivocal and presumably reflects an intrinsic tendency for methylation of CGIs. This is because, first, neuroblastomas have a much shorter history than colon cancers, and the accumulated number of methylated CGIs in neuroblastomas is expected to parallel the speed of occurrence of

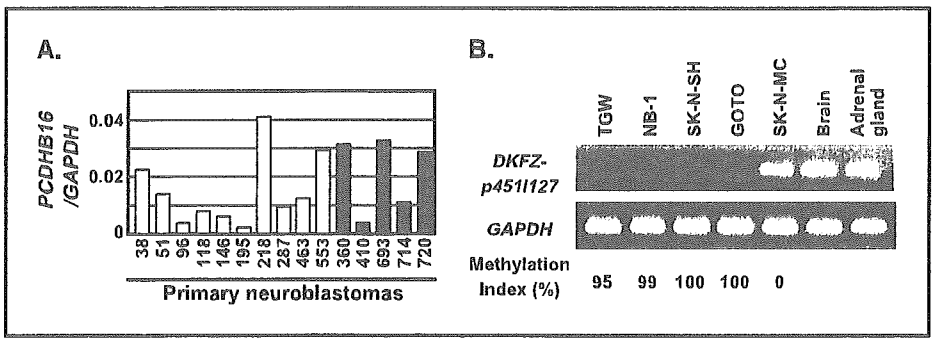

Figure 4. Effects of methylation of the *PCDHB* family and *DKFZp451I127* on gene expression. *A*, *PCDHB16* expression was analyzed by quantitative RT-PCR in 10 primary samples with low methylation (*open columns*) and five primary samples with high methylation (*closed columns*), and no difference was observed between the two groups. *B*, silencing of *DKFZp451I127* by methylation of its promoter CGI. The CGI was methylated in four cell lines, TGW, NB-1, SK-N-SH, and GOTO, whereas it was unmethylated in one cell line, SK-N-MC. *DKFZp451I127* was expressed in SK-N-MC, but not expressed at all in the four cell lines with the promoter methylation.