This has led to the initiation of RTOG 0618, a phase II study of the same SBRT regimen in patients with the same tumor characteristics but who are physiologically able to tolerate complete resection. Since December 2007, the trial has accrued approximately half of its target of 33 patients and is likely to be completed by 2010. If results are encouraging, the plan is to move forward with a randomized phase III trial of surgery versus SBRT.

Because of concerns of increased toxicity of SBRT in more centrally located tumors, the RTOG is also exploring a slightly less dose-intensive approach for tumors close to the proximal bronchial tree. RTOG 0813 is a phase I/II dose-escalation trial designed for medically inoperable patients with centrally located stage I NSCLC, with a starting dose of 50 Gy in 5 fractions. The study was activated in February 2009, with an accrual goal of 94 patients. Future plans include a randomized trial of different dose/fractionation SBRT regimens for medically inoperable early-stage lung cancer.

In locally advanced lung cancer, the RTOG completed a phase I/II study of an escalated radiation dose (74 Gy) with concurrent chemotherapy, RTOG 0117, as well as a phase II trial of escalated systemic therapy (carboplatin/paclitaxel plus cetuximab) with concurrent radiation therapy (63 Gy), RTOG 0324. In both of these studies, patients with predominantly inoperable stage IIIA/IIIB NSCLC had median survival times of 21.6 months and 22.7 months, respectively—highly promising compared with the 17-month median survival observed in RTOG 9410, the study that established concurrent chemoradiation therapy as the standard of care for locally advanced NSCLC in the RTOG. Thus, the RTOG has initiated and is the coordinating group for the Intergroup study RTOG 0617, a 4-arm phase III randomized trial of standard-dose (60 Gy) versus high-dose (74 Gy) radiation with concurrent carboplatin/paclitaxel with or without cetuximab for patients with unresectable stage IIIA/IIIB NSCLC. The study opened in November 2007 and has accrued 123 of 500 planned patients to date.

The RTOG has also coordinated a randomized phase III Intergroup study of PCI versus observation for patients without evidence of brain metastases and without progression after initial treatment for locally advanced NSCLC. RTOG 0214 closed early after failing to meet its original accrual goal of 1058 patients, but analysis of 340 randomized patients revealed a statistically significant reduction of the rate of brain metastasis from 18% to 7.7% at 1 year with PCI.[35] This was at the cost of decreased performance on a verbal learning test in the PCI group, but there was no significant difference in mini–mental status examination and quality of life. With the limited accrual, there was insufficient power to detect a difference in PFS or OS.

Protocols currently under development will focus on multimodality therapy. RTOG 0839 is a proposed study that will study carboplatin/paclitaxel/cetuximab with full-dose concurrent radiation therapy (61 Gy) as preoperative therapy for resectable stage IIIA disease with minimal N2 nodal metastasis. RTOG 0937 is a proposed randomized phase II study of consolidative radiation therapy to thoracic and limited extrathoracic sites after chemotherapy and PCI for extensive-stage SCLC. In addition, all of the currently active and proposed protocols include a translational research component to investigate the predictive and/or prognostic value of blood and/or urine biomarkers.

Southwest Oncology Group

Building on earlier work in superior sulcus (Pancoast) tumors, S0920 will explore the addition of cetuximab to the regimen studied in the recently completed S0220 but will include patients with more advanced disease (IIB, IIIA, and IIIB, including ipsilateral supraclavicular nodal disease, all T3 or T4). Enrolled patients will receive cisplatin 50 mg/m^2 on days 1, 8, 29, and 36 and etoposide 50 mg/m^2 on days 1-5 and 29-33 with concurrent thoracic radiation therapy of 54 Gy (the SWOG standard NSCLC regimen) in combination with cetuximab 250 mg/m^2 weekly after a loading dose. Patients will then proceed to surgical resection, with additional consolidation chemotherapy. The primary endpoint is the pathologic complete response rate.

Also for early-stage NSCLC, SWOG is participating in E1505 and E5597 and has other SWOG-led studies, in particular S0720, focused on personalizing adjuvant chemotherapy. S0720 is founded on work by Zheng et al,[36] as well as others[37] demonstrating that ERCC1 and RRM1 levels provide both prognostic value and predictive value for platinum-based chemotherapy in NSCLC. S0720 will test the feasibility of pharmacogenomically directed adjuvant therapy by accruing patients with completely resected stage I NSCLC (≥ 2 cm in size) and assigning therapy based on assessment of ERCC1 and RRM1 levels from the surgical specimen. The primary endpoint of S0720 is feasibility, defined by the percentage of patients who can be assigned treatment appropriately, reflecting the adequacy of tumor specimen collection and analysis.

Another early-stage NSCLC study is S0424, which is investigating the molecular epidemiology of early-stage NSCLC in smoking and nonsmoking men and women. By performing extensive tissue- and blood-based analyses on multiple pathways, this study assesses the influence of smoking, hormonal factors, and other exposures on sex differences in lung cancer. The study is nearly completed but requires more never-smoking men to finish accrual.

The major first-line NSCLC trial for SWOG is S0819, a phase III trial that will randomize patients to carboplatin/paclitaxel (plus bevacizumab in eligible patients) with or without cetuximab. The 4-drug regimen was studied in S0536 with encouraging results and promising correlate trial work.[38] SWOG's initial work with cetuximab, S0342, combined the agent with carboplatin/paclitaxel in different schedules with favorable results in the concurrent arm, especially in patients with EGFR overexpression by FISH analysis.[39] EGFR FISH analysis will be an important component of S0819, which aims to screen 1545 patients to identify adequate number of patients with EGFR expression by IHC (required for study entry) and 618 FISH-positive patients.

For patients with poor PS with advanced-stage NSCLC, S0709 will evaluate erlotinib versus erlotinib plus chemotherapy using a serum proteomics pattern suggestive of erlotinib benefit.[26] Patients on the chemotherapy arm will received carboplatin/paclitaxel on day 1 then erlotinib on days 2-16 of each 21-day cycle to allow for "pharmacodynamic separation," as previously piloted at University of California, Davis.[40]

Another agent that SWOG is studying is conatumumab (AMG 655), a proapoptotic agent that directly activates TRAIL-TR-2. S0810 will enroll 60 patients per arm who will be randomized to conatumumab 15 mg/kg every 3 weeks or the same dose plus pemetrexed

500 mg/m^2. Conatumumab is also being added to standard first-line cisplatin/pemetrexed chemotherapy either with (S0814) or without (S0813) bevacizumab with maintenance conatumumab in both studies after completion of 6 cycles of chemotherapy. S0814 will also include maintenance bevacizumab. The accrual goal is 70 patients per trial.

In SCLC, SWOG is looking at a trial of large-volume chemoradiation for limited-stage disease (S0908), and in extensive-stage disease, cediranib will be combined with cisplatin/etoposide in a randomized phase III study, S0938. S0938 will enroll 600 patients who will be randomized to standard cisplatin/etoposide chemotherapy with or without cediranib (which will be continued as a single agent after completion of 4 cycles of chemotherapy), with an OS endpoint. The study has embedded marker validation with a biomarker-embedded design. Aflibercept (VEGF Trap) and topotecan combination therapy will be studied for recurrent SCLC in S0802.

Patients with newly diagnosed mesothelioma will be eligible for S0905, which will randomize patients to cisplatin/pemetrexed with cediranib or placebo after completion of the phase I dose-escalation period. This study is building on encouraging single-agent activity of second-line cediranib for mesothelioma in the S0509 study presented at the ASCO 2009 annual meeting.[41] Also in mesothelioma, S0722 is exploring the use of everolimus (RAD001).

Conclusion

The cooperative group system plays a vital role in the advancement of therapy for NSCLC, SCLC, and mesothelioma. The cooperative groups of North America have been pivotal in showing benefit with the anti-VEGF agent bevacizumab and the anti-EGFR drug erlotinib, as well as the importance of adjuvant chemotherapy among other advances. As outlined in this article, the portfolios of the groups and international cooperative groups are full of varied and important studies. The use of an antiangiogenesis approach, as seen in E1505 with adjuvant bevacizumab, in BR.29 with first-line cediranib, and in multiple other trials incorporating anti-VEGFR TKIs, is a primary focus. The groups are also very involved in investigating therapies in the EGFR pathway, including cetuximab, and better understanding of how to use erlotinib and gefitinib in patients with activating mutations and other indicators of benefit such as proteomic profiles.

The cooperative groups provide a mechanism for asking critical questions such as the value of postoperative radiation therapy and the role of lesser resections for smaller stage I tumors that would be very difficult in any other setting. One of the incredible strengths of the cooperative groups is the breadth and depth of translational science possible within trials. This translational strength is seen in the multitude of trials currently active or in development looking at real-time sample analysis of EGFR mutations, RRM1 expression, and others in the move toward personalized therapy. Multiple other targeted agents, including those targeting IGF-1R, Hedgehog, and direct inducers of apoptosis are also in trials within the cooperative group system. Within the context of the cooperative group system, the future of thoracic malignancy therapy looks promising.

Disclosures

Heather Wakelee has received research funding from Bayer Pharmaceuticals Corporation; Eli Lilly and Company; Genentech, Inc.; Novartis Pharmaceuticals Corporation; and Pfizer Inc.

Nagahiro Saijo holds stock or equity ownership in Takeda Pharmaceuticals.

Joan Schiller has received research funding from Celgene Corporation; Genentech, Inc.; Geron; Merck & Co., Inc.; Novartis Pharmaceuticals Corporation; Pfizer Inc.; and Schering-Plough Corporation and has served as a paid consultant or been on the Advisory Board of AstraZeneca; Bayer Pharmaceuticals Corporation; Biomira; Celgene Corporation; Cell Genesys, Inc.; Eli Lilly and Company; Genentech, Inc.; Genomic Health, Inc.; GlaxoSmith-Kline; ImClone Systems Incorporated; Merck & Co., Inc.; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Roche Pharmaceuticals; Sunesis Pharmaceuticals, Inc.; and Telik, Inc.

Bill Loo Jr. has served as a paid consultant or been on the Advisory Board of CyberHeart, Inc. and is a member of the Speaker's Bureau for Accuray Incorporated; General Electric Company; and superDimension, Ltd.

David R. Gandara has served on the Board of Directors or held other leadership positions with Response Genetics, Inc.; has received research funding from Abbott Laboratories, Bristol-Myers Squibb Company, and Eli Lilly and Company; and has served as a paid consult or been on the Advisory Board of AstraZeneca, Bayer Pharmaceuticals Corporation, Bristol-Myers Squibb Company, Genentech, Inc., Pfizer Inc., and sanofi-aventis U.S.

Martin J. Edelman has received research funding from Bristol-Myers Squibb Company, Eli Lilly and Company, Schering-Plough Corporation, and Tragara Pharmaceuticals Inc. and has served as paid consultant or been on the Advisory Board of Bristol-Myers Squibb Company; Eli Lilly and Company; Facet Biotech Corporation; Genentech, Inc.; and Tragara Pharmaceuticals Inc.

Glenwood Goss has received research funding from AstraZeneca and Ortho Biotech Products, L.P. and has served as a paid consultant or been on the Advisory Board of AstraZeneca, Pfizer Inc., and Roche Pharmaceuticals.

Alex Adjei has received research funding from Ardea Biosciences, Inc. and Eli Lilly and Company.

Everett E. Vokes, Joe Bill Putnam Jr., and Kemp H. Kernstine have no relevant relationships to disclose.

References

1. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. *BMJ* 1995; 311:899-909.
2. Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. *J Clin Oncol* 2004; 22:330-53.
3. Socinski MA, Crowell R, Hensing TE, et al. Treatment of non-small cell lung cancer, stage IV: ACCP evidence-based clinical practice guidelines (2nd edition). *Chest* 2007; 132:277S-89S.
4. Pisters KM, Evans WK, Azzoli CG, et al. Cancer Care Ontario and American Society of Clinical Oncology Adjuvant Chemotherapy and Adjuvant Radiation Therapy for Stages I-IIIA Resectable Non Small-Cell Lung Cancer Guideline. *J Clin Oncol* 2007; 25:5506-18.
5. National Comprehensive Cancer Network [Web site]. Available at: http://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf. Accessed: November 24, 2007.
6. Ettinger DS, Bepler G, Bueno R, et al. Non-small cell lung cancer clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 2006; 4:548-82.
7. Delbaldo C, Michiels S, Syz N, et al. Benefits of adding a drug to a single-agent or a 2-agent chemotherapy regimen in advanced non-small-cell lung cancer: a meta-analysis. *JAMA* 2004; 292:470-84.
8. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006; 355:2542-50.
9. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet* 2009; 373:1525-31.

10. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005; 353:123-32.
11. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. *N Engl J Med* 2005; 352:2589-97
12. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361:947-57.
13. Kobayashi K, Inoue A, Maemondo M, et al. First-line gefitinib versus first-line chemotherapy by carboplatin (CBDCA) plus paclitaxel (TXL) in non-small cell lung cancer (NSCLC) patients (pts) with EGFR mutations: a phase III study (002) by North East Japan Gefitinib Study Group. *J Clin Oncol* 2009; 27(15 suppl):411s (Abstract 8016).
14. Strauss GM, Herndon JE, Maddaus MA, et al. Adjuvant chemotherapy in stage IB non-small cell lung cancer (NSCLC): update on Cancer and Leukemia Group B (CALGB) protocol 9633. *J Clin Oncol* 2006; 24(18 suppl):365s (Abstract 7007).
15. Strauss GM, Herndon J, Maddaus MA, et al. Randomized Clinical Trial of adjuvant chemotherapy with paclitaxel and carboplatin following resection in Stage IB non-small cell lung cancer (NSCLC): report of Cancer and Leukemia Group B (CALGB) Protocol 9633. *J Clin Oncol* 2004; 22(14 suppl):621s (Abstract 7019).
16. Strauss GM, Herndon JE II, Maddaus MA, et al. Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups. *J Clin Oncol* 2008; 26:5043-51.
17. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. *Ann Thorac Surg* 1995; 60:615-22, discussion 622-3
18. Potti A, Mukherjee S, Petersen R, et al. A genomic strategy to refine prognosis in early-stage non-small-cell lung cancer. *N Engl J Med* 2006; 355:570-80.
19. Govindan R, Bogart J, Wang X, et al. Phase II study of pemetrexed, carboplatin, and thoracic radiation with or without cetuximab in patients with locally advanced unresectable non-small cell lung cancer: CALGB 30407. *J Clin Oncol* 2009; 27(15 suppl):383s (Abstract 7505).
20. Edelman MJ, Watson D, Wang X, et al. Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy--Cancer and Leukemia Group B Trial 30203. *J Clin Oncol* 2008; 26:848-55.
21. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* 2005; 23:5892-9
22. Socinski MA, Novello S, Brahmer JR, et al. Multicenter, phase II trial of sunitinib in previously treated, advanced non-small-cell lung cancer. *J Clin Oncol* 2008; 26:650-6.
23. Schiller JH, Dahlberg SE, Mehra M, et al. A phase III trial of carboplatin, paclitaxel, and thoracic radiation therapy with or without thalidomide in patients with stage III non-small cell carcinoma of the lung (NSCLC): E3598. *J Clin Oncol* 2009; 27(15 suppl):382s (Abstract 7503).
24. Fidias PM, Dakhil SR, Lyss AP, et al. Phase III study of immediate compared with delayed docetaxel after front-line therapy with gemcitabine plus carboplatin in advanced non-small-cell lung cancer. *J Clin Oncol* 2009; 27:591-8.
25. Belani CP, Brodowicz T, Ciuleanu T, et al. Maintenance pemetrexed (Pem) plus best supportive care (BSC) versus placebo (Plac) plus BSC: a randomized phase III study in advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* 2009; 27(15 suppl):407s (Abstract CRA8000).
26. Taguchi F, Solomon B, Gregorc V, et al. Mass spectrometry to classify non-small-cell lung cancer patients for clinical outcome after treatment with epidermal growth factor receptor tyrosine kinase inhibitors: a multicohort cross-institutional study. *J Natl Cancer Inst* 2007; 99:838-46.
27. Douillard JY, Rosell R, Delena M, et al. ANITA: phase III adjuvant vinorelbine (N) and cisplatin (P) versus observation (OBS) in completely resected (stage I-III) non-small-cell lung cancer (NSCLC) patients (pts): final results after 70-month median follow-up. On behalf of the Adjuvant Navelbine International Trialist Association. *J Clin Oncol* 2005; 23(16 suppl):624s (Abstract 7013).
28. Lally BE, Zelterman D, Colasanto JM, et al. Postoperative radiotherapy for stage II or III non-small-cell lung cancer using the surveillance, epidemiology, and end results database. *J Clin Oncol* 2006; 24:2998-3006.
29. Van Schil PE, Baas P, Gaafar R, et al. Phase II feasibility trial of induction chemotherapy (ICT) followed by extrapleural pneumonectomy (EPP) and postoperative radiotherapy (PORT) for cT3N1M0 or less malignant pleural mesothelioma (MPM) (EORTC 08031). *J Clin Oncol* 2009; 27(15 suppl):384s (Abstract 7509).
30. Hamada C, Ohta M, Wada H, et al. Survival benefit of oral UFT for adjuvant chemotherapy after completely resected non small-cell lung cancer. *J Clin Oncol* 2004; 22(14 suppl):617s (Abstract 7002).
31. Satouchi M, Yamamoto N, Chiba Y, et al. Randomized phase III study of mitomycin/vindesine/cisplatin (MVP) versus weekly irinotecan/carboplatin (IC) or weekly paclitaxel/carboplatin (PC) with concurrent thoracic radiotherapy (TRT) for unresectable stage III non-small cell lung cancer (NSCLC): WJTOG0105. *J Clin Oncol* 2009; 27(15 suppl):383s (Abstract 7504).
32. Vincent MD, Butts C, Seymour L, et al. Updated survival analysis of JBR.10: a randomized phase III trial of vinorelbine/cisplatin versus observation in completely resected stage IB and II non-small cell lung cancer (NSCLC). *J Clin Oncol* 2009; 27(15 suppl):382s (Abstract 7501).
33. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005; 366:1527-37
34. Kelly K, Gaspar LE, Chansky K, et al. Low incidence of pneumonitis on SWOG 0023: a preliminary analysis of an ongoing phase III trial of concurrent chemoradiotherapy followed by consolidation docetaxel and Iressa/placebo maintenance in patients with inoperable stage III non-small cell lung cancer. *J Clin Oncol* 2005; 23(16 suppl):634s (Abstract 7058).
35. Gore EM, Bae K, Wong S, et al. A phase III comparison of prophylactic cranial irradiation versus observation in patients with locally advanced non-small cell lung cancer: initial analysis of Radiation Therapy Oncology Group 0214. *J Clin Oncol* 2009; 27(15 suppl):383s (Abstract 7506).
36. Zheng Z, Chen T, Li X, et al. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. *N Engl J Med* 2007; 356:800-8.
37. Lord RV, Brabender J, Gandara D, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. *Clin Cancer Res* 2002; 8:2286-91.
38. Gandara D, Kim ES, Herbst RS, et al. S0536: carboplatin, paclitaxel, cetuximab, and bevacizumab followed by cetuximab and bevacizumab maintenance in advanced non-small cell lung cancer (NSCLC): a SWOG phase II study. *J Clin Oncol* 2009; 27(15 suppl):410s (Abstract 8015).
39. Hirsch FR, Herbst RS, Olsen C, et al. Increased EGFR gene copy number detected by fluorescent in situ hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy. *J Clin Oncol* 2008; 26:3351-7.
40. Davies AM, Ho C, Beckett L, et al. Intermittent erlotinib in combination with pemetrexed: phase I schedules designed to achieve pharmacodynamic separation. *J Thorac Oncol* 2009; 4:862-8.
41. Garland LL, Chansky K, Wozniak A, et al. SWOG S0509: a phase II study of novel oral antiangiogenic agent AZD2171 (NSC-732208) in malignant pleural mesothelioma. *J Clin Oncol* 2009; 27(15 suppl):384s (Abstract 7511).

Jpn J Clin Oncol 2009;39(12)784–790
doi:10.1093/jjco/hyp102

A Phase II Study of Cisplatin and Irinotecan as Induction Chemotherapy Followed by Accelerated Hyperfractionated Thoracic Radiotherapy with Daily Low-dose Carboplatin in Unresectable Stage III Non-small Cell Lung Cancer: JCOG 9510

Tomoki Fujii[1], Hiroshi Kunikane[1], Hiroaki Okamoto[1], Koshiro Watanabe[1], Hideo Kunitoh[2], Kiyoshi Mori[3], Akira Yokoyama[4], Haruhiko Fukuda[5], Tomohide Tamura[6] and Nagahiro Saijo[7]

[1]Department of Respiratory Medicine and Medical Oncology, Yokohama Municipal Citizen's Hospital, Kanagawa, [2]Department of Respiratory Medicine, Mitsui Memorial Hospital, Tokyo, [3]Department of Internal Medicine, Tochigi Prefectural Cancer Center, Tochigi, [4]Department of Internal Medicine, Niigata Cancer Center Hospital, Niigata, [5]Japan Clinical Oncology Group Data Center, Center for Cancer Control and Information Services, National Cancer Center, [6]Department of Thoracic Oncology, National Cancer Center Hospital Central, Tokyo and [7]Department of Medical Oncology, Kinki University School of Medicine, Osaka, Japan

Received May 7, 2009; accepted July 29, 2009; published online September 20, 2009

Objective: It is important to find optimal regimens of cisplatin (CDDP)-based third-generation chemotherapy and radiotherapy for patients with unresectable Stage III non-small cell lung cancer (NSCLC).
Methods: This Phase II study was designed to determine the toxicity and efficacy of two courses of chemotherapy (CDDP 80 mg/m^2 on day 1 and irinotecan 60 mg/m^2 on days 1 and 8) followed by accelerated hyperfractionated thoracic radiotherapy (60 Gy/40 fractions in 4 weeks) combined with daily carboplatin (CBDCA) administration. CBDCA was administered at a target area under the plasma level–time curve of 0.4 × (24 h creatinine clearance + 25), according to Calvert's formula.
Results: Twenty-six patients were enrolled in the study. The patients' median age was 63 years (range 40–74 years) and included 22 males and 4 females. Seven patients were Stage IIIA and 19 were Stage IIIB. Twenty had a performance status (PS) of 1 versus six with a PS of 0. There was one treatment-related death due to sepsis and pneumonia associated with Grade 4 neutropenia and diarrhea during chemotherapy. Grade 3 or 4 neutropenia and diarrhea were observed in 14 and 5 patients, respectively. Toxicity of the radiotherapy was mild. There were 0 complete response and 13 partial responses, giving a response rate of 50.0%. Median survival time and 2-year survival were 16.4 months and 21.5%, respectively. This study was designed with Simon's two-stage design, and the response rate did not meet the criteria to proceed to the second stage and the study was terminated early.
Conclusions: This regimen might be inactive for patients with unresectable Stage III NSCLC.

Key words: cisplatin – irinotecan – carboplatin – chemoradiotherapy – non-small cell lung cancer

INTRODUCTION

Over the past 2 decades, a great number of clinical trials have gradually proven the benefits of a chemotherapeutic approach for treatment of unresectable non-small cell lung cancer (NSCLC) (1,2). In unresectable Stage III NSCLC, in which the tumor is apparently confined to the chest but is surgically unresectable, several randomized trials have shown that combinations of chemotherapy and thoracic radiotherapy have improved survival compared with radiotherapy alone (3–6). It is important to find optimal regimens of combined chemotherapy and radiotherapy and to evaluate the feasibility and efficacy of those combinations.

For reprints and all correspondence: Hiroaki Okamoto, Department of Respiratory Medicine and Medical Oncology, Yokohama Municipal Citizen's Hospital, 56 Okazawa-cho, Yokohama 240-8555, Japan.
E-mail: scyooka@alles.or.jp

Irinotecan (CPT-11) is an antitumor agent which inhibits the nuclear enzyme topoisomerase I (7,8). CPT-11 has played a significant role in the development of chemotherapy for NSCLC since the initial reports of its efficacy as a single agent (9,10). Combination chemotherapy of CPT-11 and cisplatin (CDDP), which is also a commonly used agent for NSCLC, is a promising regimen for NSCLC, as its high antitumor activity and manageable toxicity have been reproducibly reported (11,12). One critical but uncommon toxicity of CPT-11 is reported to be pulmonary toxicity (10), and it is necessary to clarify how the chemotherapy regimen should be combined with thoracic radiotherapy in patients with Stage III NSCLC.

In addition to combined radio-chemotherapy, concomitant treatment with low doses of radiosensitizers has also been investigated in patients with Stage III NSCLC. Schaake-Koning et al. (13) reported that daily low-dose CDDP combined with thoracic radiation improved the local control of tumors in a randomized study. Furthermore, its favorable results were also confirmed in another Phase II study (14). Carboplatin (CBDCA) has also been investigated as a radiosensitizer (15). It has been suggested that CBDCA may be superior to CDDP in this role because it would provide a greater platinum concentration within cells at the time of irradiation (16). We have reported the concurrent daily CBDCA (25 mg/m^2) and accelerated hyperfractionated thoracic radiotherapy (AHRT) in locally advanced NSCLC (17). Of the 31 patients, the response rate was 84% (26/31) and the median survival time (MST) was 9.8 months. Major acute toxicity (Grade $\geq$3) included 55% with leukopenia, 16% with thrombocytopenia and 23% with esophagitis. Area under the plasma level–time curve (AUC) of CBDCA was significantly correlated with efficacy and leukopenia. In this setting, we concluded that daily CBDCA AUC of 0.4 plus concurrent AHRT was the most effective and safe treatment in locally advanced NSCLC.

On the other hand, the CDDP plus CPT-11 regimen is one of the standard platinum-based combination chemotherapies including a new agent in Stage IIIB/IV NSCLC in Japan (11). Therefore, in order to improve therapeutic outcome in patients with unresectable Stage III NSCLC, we have conducted a Phase II study of a regimen of two courses of CDDP plus CPT-11 as an induction chemotherapy, followed by AHRT with daily low-dose CBDCA administration.

PATIENTS AND METHODS

Patient Selection

Patients with histologically or cytologically confirmed unresectable Stage III NSCLC who had not received cancer therapy were enrolled in this study. Staging for entry criteria was performed according to the lung cancer staging system of the International Union against Cancer. Staging procedures included chest X-ray, computed tomography (CT) scan of the chest, CT scan or magnetic resonance imaging of the brain, CT scan or ultrasound of the abdomen and isotope bone scanning. N-status was mainly based on size criteria in chest CT scan. Patients with pleural or pericardial effusion were excluded from the study. Each patient was required to meet the following criteria: Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; <75 years of age; predicted area of radiation field is less than half of one lung; adequate hematological, pulmonary, renal and hepatic function, i.e. white blood cell (WBC) count $\geq$4000/μL, hemoglobin level $\geq$10 g/dl, platelet count $\geq$130 000/μL, PaO_2 $\geq$70 torr, blood urea nitrogen and serum creatinine level no higher than the upper limit of normal, creatinine clearance (Ccr) $\geq$60 ml/min, serum total bilirubin level $\leq$1.5 mg/dl and serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels less than twice the upper limit of normal.

Patients with uncontrolled heart failure or infection, chronic pulmonary disease which restricts thoracic radiation, prolonged diarrhea, ileus, gastrointestinal bleeding or history of myocardial infarction in the last 3 months were excluded from the study. Female patients in pregnancy or lactation during chemotherapy were also excluded. All patients were required to give their own written informed consent.

Treatment Schedule

After enrollment in the study, the patients received chemotherapy consisting of intravenous infusion of 80 mg/m^2 of CDDP on day 1 and 60 mg/m^2 of CPT-11 on days 1 and 8. The chemotherapy was repeated 3–4 weeks after the start of the first course, as long as the patients had sufficiently recovered from toxicity. The chemotherapy was to be performed for two courses, unless unacceptable toxicity or disease progression occurred.

Four weeks after the start of the second course of chemotherapy, thoracic radiotherapy was started. The initial opposing anterior–posterior treatment fields encompassed the primary tumor, the bilateral mediastinal lymph nodes and the ipsilateral hilar nodes. The supraclavicular nodes were included within the field when there was clinical evidence of their involvement. A 1.5 cm tumor-free margin was required. The fraction size delivered was 1.5 Gy, given twice per day, 5 days per week. Thus, the total radiation dose was 60 Gy in 40 fractions over 4 weeks. The methods for spinal block and boost after the first 30 Gy delivery was left to the discretion of the treating radiation oncologist. On each day of thoracic radiotherapy, the patients also received intravenous CBDCA. CBDCA was dosed to a target AUC of 0.4 × (24 h Ccr + 25), according to Calvert's formula (18), and was administered intravenously over 15 min immediately before the first radiation of the day. The CBDCA AUC of 0.4 was determined based on our previous study (17).

CPT-11 on day 8 was skipped if the WBC count was <3000/μL, platelet count <75 000/μL or Grade 2 or higher diarrhea or abdominal pain was seen. During chemotherapy, if the WBC count fell <2000/μL or the neutrophil count

dropped $<1000/\mu L$, daily granulocyte colony-stimulating factor (G-CSF) was administered subcutaneously until the WBC count increased to $\geq 10\,000/\mu L$ or was no longer clinically indicated. Radiotherapy and concomitant use of G-CSF was contraindicated. When the second course of CDDP plus CPT-11 was started, each patient was required to meet the following criteria: WBC count $\geq 4000/\mu L$, neutrophil count $\geq 2000/\mu L$, platelet count $\geq 130\,000/\mu L$, serum creatinine level ≤ 1.5 mg/dl, serum GOT and GPT levels Grade 0 or 1, Ccr ≥ 30 ml/min, body temperature $<38.0^{\circ}C$ and PS 0, 1 or 2. For patients receiving G-CSF, 3 days after discontinuation, patients were required to meet the aforementioned hematological toxicity criteria prior to starting the second course of CDDP plus CPT-11. If the second course was delayed 2 weeks or more due to toxicity, chemotherapy with CDDP plus CPT-11 and low-dose CBDCA was terminated and only radiotherapy was used. According to toxicities in the first course of chemotherapy, the dose of CDDP was reduced by 25% for Grade 4 leukopenia, Grade 4 neutropenia ≥ 7 days, Grade 3 thrombocytopenia, Grade 3 or 4 mucositis or Grade 2 or higher renal toxicity, and by 50% for Grade 4 thrombocytopenia. The dose of CPT-11 was reduced by 25% for Grade 3 or 4 diarrhea and administration of CPT-11 was terminated if Grade 2 or higher pulmonary toxicity was seen.

Criteria for starting AHRT with daily low dosage CBDCA administration were the same as mentioned above for the second course of CDDP plus CPT-11. Six weeks after initiation of the second course of chemotherapy, if the same criteria were not fulfilled, CBDCA administration was terminated. In that case, only radiotherapy was used.

During chemoradiation, if the WBC count fell $<2000/\mu L$, neutrophil count $<1000/\mu L$ or platelet count $<50\,000/\mu L$, daily use of CBDCA was suspended and only radiotherapy was continued. After recovery from neutropenia, administration of CBDCA was restarted. In case of Grade 4 hematological toxicities, chemoradiation was to be terminated. However, if any toxicity improved Grade 2 or lower, only radiotherapy could be used. If the PaO_2 level decreased by 10 torr or more compared with baseline value, chemoradiation was suspended and if it returned to baseline, treatment could be started again carefully. If Grade 3 or 4 radiation-related esophagitis was seen, chemoradiation was suspended but could be started again when this toxicity improved to Grade 2 or lower. If patients had a fever of 38°C or higher, chemoradiation was suspended until they were afebrile. Chemoradiation was also suspended when deterioration of PS to 3 or 4 occurred, and PS 0, 1 or 2 was necessary to restart the protocol treatment.

Treatment Evaluation

Tumor response and toxicity were evaluated according to World Health Organization response criteria (19) and Japan Clinical Oncology Group (JCOG) toxicity criteria (20), respectively. Complete response (CR), partial response (PR) and no change (NC) were reviewed and confirmed by central review with chest radiographs or CTs at the regular disease-group meeting. Complete blood cell count and routine blood chemistry were checked twice a week, and arterial blood gas and chest radiographs were checked at least once a week, until the patient had apparently recovered from all acute toxic effects after the completion of the treatment. In this trial, the methods to follow-up the patient after the protocol treatment were not clearly defined. In addition, not only late toxicities but also recurrence patterns after finishing protocol treatment were not routinely recorded in the case report form (CRF). Therefore, the interval of evaluation for late toxicities was left to the discretion of the treating physician. Consequently, the frequency of visiting the doctors and radiologic examinations was heterogeneous among the patients.

Study Design and Statistical Methods

This trial was designed as a multicenter prospective single-arm Phase II study, and the study protocol was approved by the Clinical Trial Review Committee (protocol review committee) of JCOG (21) and the institutional review board of each participating institution before study activation. After pre-treatment staging and eligibility evaluation, patients were registered at the JCOG Data Center by telephone or fax. The study was performed by the JCOG Lung Cancer Study Group and all study data were managed by the JCOG Data Center.

The primary endpoints of this study were the overall response rate (ORR) and overall survival (OS). The ORR was defined as the proportion of the patients with CR or PR out of all eligible patients. The confidence intervals for the ORR were calculated based on the exact method. The OS was measured from the date of patient registration to the date of death due to any cause. If a patient was alive at the final follow-up survey, OS was censored at the last contact date. The estimates of survival distribution were calculated by the Kaplan–Meier method and confidence intervals were based on Greenwood's formula (22). And 2-year OS was expected to be ~40%. The progression-free survival was not measured in this study.

We set an expected level (P1) of response rate as 80%, threshold level (P0) as 60%, α-error level was 0.05 and β-error level was 0.10. We set the planned total sample size as 45 according to Simon's minimax two-stage design (23). If 15 or fewer patients out of 26 patients showed objective responses at the first stage, the study was to be terminated early. The OS was followed up to 20 months after the last enrollment.

RESULTS

Patient Characteristics

Between February 1996 and January 1999, 26 patients from 5 institutions were enrolled in this study and all received induction chemotherapy. The pace of enrollment was approximately one-fourth of the planned one in the protocol.

For the pre-specified first stage decision, the accrual was temporarily closed and the response rate was assessed. Characteristics of the 26 patients are listed in Table 1. The patients included 22 men and 4 women, with a median age of 63 (range, 40–74) years. The histologic classifications included adenocarcinoma in 14 patients and squamous cell carcinoma in 12. Seven patients were in Stage IIIA and 19 were in Stage IIIB. Six patients had ECOG PS of 0 and 20 had that of 1. All of the 26 patients were eligible and evaluable for both tumor response and toxicity.

Treatment Delivery and Protocol Compliance

Of the 26 patients enrolled in the study, 15 completed both of the scheduled chemotherapy and radiotherapy. Protocol compliance in the 26 patients is summarized in Tables 2 and 3. In six patients, treatment was terminated after the first course of chemotherapy. The reasons for the withdrawal were disease progression in three patients and toxicity in three. In three patients with disease progression after the first course of CDDP plus CPT-11, one patient could receive sequential chemoradiation. In one patient Cre >1.5 mg/dl persisted, whereas in another patient, Grade 4 diarrhea, Grade 2 neutropenia and Grade 2 fever caused deterioration of PS and resulted in termination of induction chemotherapy. That patient died of sepsis and pneumonia from Grade 4 neutropenia and diarrhea which we categorized as treatment-related death. One patient had disease progression after two courses of chemotherapy and could not receive radiotherapy. One patient experienced Grade 4 leukopenia and the dose of CDDP in the second course should have been reduced to 75% of the original dosage. However, this patient received only CPT-11 and CDDP was improperly omitted in the second course, which was judged as a protocol violation. Delay in the start of the second course occurred in three patients. CPT-11 administration on day 8 was skipped in four patients and three patients had dose reduction of CPT-11 in the second course. The reason for dose omission or dose reduction was diarrhea in five patients.

Twenty patients received thoracic radiotherapy according to the protocol but 3 of the 20 patients could not receive the whole 60 Gy of radiation with daily CBDCA because of hypoxemia, emesis or onset of herpes zoster in the radiation field in each patient, respectively. Radiotherapy could not be delivered for six patients. The reason for not receiving radiotherapy was disease progression in four patients and toxicity in two patients including treatment-related death in one patient. Of the 20 patients receiving radiotherapy, actual mean radiation dose and actual mean number of CBDCA infusion was 56.8 Gy and 17.5 times, respectively (Table 3).

Table 1. Patient characteristics

Characteristics		No.	%
Age (years)			
Median	63		
Range	40–74		
Sex			
Male		22	84.6
Female		4	15.4
Histology			
Adenocarcinoma		14	53.8
Squamous cell carcinoma		12	46.2
Others		0	0
Clinical Stage			
Stage IIIA		7	26.9
Stage IIIB		19	73.1
T-stage			
T1		4	15.4
T2		6	23.1
T3		5	19.2
T4		11	42.3
N-stage			
N0		2	7.7
N1		2	7.7
N2		11	42.3
N3		11	42.3
Performance status (ECOG)			
0		6	23.1
1		20	76.9

ECOG, Eastern Cooperative Oncology Group.

Table 2. Dose intensity of chemotherapy phase ($n = 26$)

	Planned DI	Actual DI	%[a]
CDDP	26.7	23	86
CPT-11	40	33.3	83

DI, dose intensity (mg/m^2/week); CDDP, cisplatin; CPT-11, irinotecan.
[a]Percentage of the drug dose actually delivered, vs. the planned dose, is presented.

Table 3. Chemoradiation delivery ($n = 20$)

	Planned delivery	Actual delivery, mean
AHRT	60 Gy	56.8 Gy
CBDCA infusion	20 times	17.5 times

AHRT, accelerated hyperfractionated thoracic radiotherapy; CBDCA, carboplatin.

Toxicity

There was one treatment-related death due to septic shock and pneumonia associated with Grade 4 neutropenia, Grade 4 thrombocytopenia and Grade 4 diarrhea. That patient had CDDP and CPT-11 administration on day 1 and CPT-11 on day 8 in the first course and suffered from serious toxicity. *Pseudomonous aeruginosa* was detected in the microbiological culture test from the stool of the patient. This patient died on day 35 from toxicities mentioned above. Toxicities in the 26 patients are listed in Table 4. Grade 3 or 4 neutropenia occurred in 54% of the patients. Grade 3 or 4 thrombocytopenia occurred in four patients and one patient required platelet transfusion.

The most frequent non-hematological toxicity was diarrhea, and Grade 2 or more occurred in 46% of the patients. Five patients had Grade 2 esophagitis during radiotherapy but it did not cause termination of the therapy. Pulmonary toxicity was not evident during the radiotherapy, as well as CPT-11 including chemotherapy. In one patient, radiotherapy was terminated due to a decrease in arterial oxygen pressure by 17 torr when compared with baseline but that patient also had disease progression during the therapy and it was difficult to evaluate the causal relationship to the protocol treatment. In this trial, late toxicities after finishing protocol treatment were not routinely recorded in CRF.

Table 4. Toxicity in 26 patients (JCOG grade)

	0	1	2	3	4
Leukopenia	4	5	10	7	0
Neutropenia	4	2	6	8	6
Anemia	2	4	13	7	—
Thrombocytopenia	16	5	1	3	1
Bilirubin	22	—	3	1	0
GOT	18	7	1	0	0
GPT	10	11	3	2	0
ALP	19	7	0	0	0
Creatinine	21	4	1	0	0
Arterial oxygen pressure	5	18	3	0	0
Hypo/hypernatremia	9	12	4	1	0
Hypo/hyperkalemia	23	1	1	1	0
Emesis	1	13	11	1	—
Cardiac dysfunction	24	1	0	0	1
Proteinuria	22	4	0	0	0
Hematuria	21	5	0	0	0
Diarrhea	3	11	7	3	2
Esophagitis	8	13	5	0	0
Fever	20	3	3	0	0
Weight loss	8	9	8	1	—

JCOG, Japan Clinical Oncology Group; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaliphosphatase.

Response and Survival

Objective tumor response is summarized in Table 5. Among the 26 patients, there were 13 PRs and 0 CR, giving a response rate of 50% (95% confidence interval, 30–70%). In 10 patients, a PR was achieved before the start of radiotherapy. Disease progression occurred during chemotherapy in four patients, who had to terminate the protocol treatment. Tumor response could not be evaluated in the patient with treatment-related death. The response rate at the first stage did not meet the criteria to proceed to the second stage and the study was terminated early. Figure 1 shows the OS curve of all patients enrolled in the study. After follow-up for 20 months after the last enrollment, the MST was 16.4 months. The 1- and 2-year survival rates in the 26 patients were 65.4% and 21.5%, respectively.

DISCUSSION

The findings of the present study suggest several important points that should be applied in future studies of Stage III NSCLC, although the response rate of this combination therapy was not as high as expected. First, the protocol regimen may not be sufficiently optimized in order to keep high compliance. The inferior tumor response and the high frequency of disease progression during the induction chemotherapy with CPT-11 and CDDP appeared to be the major reason for the disappointing results, which led to the early termination of the present study. Only 10 out of the 26 patients showed >50% tumor reduction during chemotherapy. It appeared unsatisfactory when one considers

Table 5. Clinical response to the therapy in 26 patients

CR	PR	NC	PD	NE	% of CR + PR (95% confidence interval)
0	13	5	7	1	50.0 (29.9–70.1)

CR, complete response; PR, partial response; NC, no change; PD, progressive disease; NE, not evaluable.

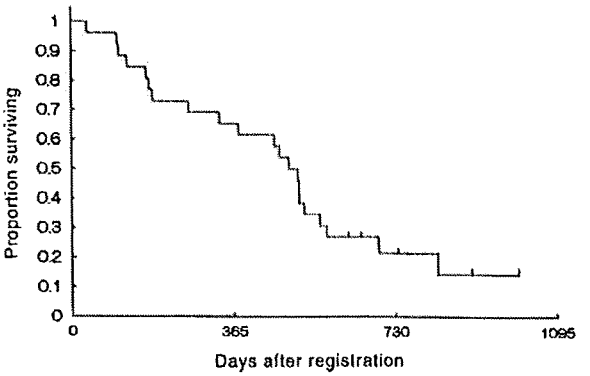

Figure 1. The overall survival curve of all patients enrolled in the study.

that only patients in Stage III were enrolled in the study. Another reason may be the fact that there were comparatively more Stage IIIB patients than Stage IIIA. Although the proportion of Stage IIIA cases was only 26.9% in this trial, in two recent studies, it was 43% and 49% (24,25). This case distribution might have contributed to the poor outcome of this study.

In the view of toxicity management, diarrhea is considered to be key toxicity to be managed carefully in combination chemotherapy using CPT-11. Relative dose intensity of CDDP, CPT-11 and radiotherapy was acceptable in this protocol; however, severe diarrhea caused lowering protocol compliance probably because high-dose loperamide therapy (26) even in the case of severe diarrhea was not used during initial period in this study. It might be possible that the anti-diarrhea agent was inadequate and protocol treatment could not be completed in some cases as a result. Had high-dose loperamide therapy been applied appropriately in all eligible cases, better response rate and survival might have been achieved in this study.

It is noteworthy that the strong association between CPT-11 delivery and antitumor response was seen in the present study. In fact, among the 12 patients who had two courses of induction chemotherapy without any delay, omission or dose reduction in CPT-11 administration, 7 showed >50% tumor reduction during the induction chemotherapy and 9 eventually achieved PR after the whole course of therapy (data not shown). This result suggests the possibility that the schedule of CPT-11 administration in this study (days 1 and 8) which was different from the more common regimen (days 1, 8 and 15) may explain the relatively low response rate and the large number of patients with disease progression. Six patients could not receive the protocol radiotherapy because of disease progression or toxicity of the induction chemotherapy. Planned omission of CPT-11 administration on day 15 was intended to reduce risk of pulmonary toxicity during radiotherapy but it might cause unsatisfactory tumor response in the chemotherapy.

Second, the timing of combination of thoracic radiation with chemotherapy may also not be optimized. The present study adopted sequential radiation following induction chemotherapy with CPT-11 and CDDP but suggests that inferior antitumor activity in the chemotherapy could cause failing to receive radiotherapy in some patients. It is difficult to find the best regimen using CPT-11 in the combined modality treatment for Stage III NSCLC.

Because late toxicities were not fully evaluated, the occurrence of both pneumonitis and delayed esophagitis might be possibly underestimated in this study. However, despite the high radiation dose, acute esophagitis were very mild contrary to our expectation, although we cannot clearly explain the reason. Most patients who could proceed to chemoradiotherapy could complete the scheduled radiation with acceptable toxicity. The MST of 16.4 months in the present study was almost as good as in other studies that showed high response rates and survival benefit in Stage III NSCLC. Although our study was prematurely closed after interim analysis because of low response rate, OS which was one of the primary endpoints was comparable with other literatures (24,25,27). In our opinion, AHRT with CBDCA still remains a chemoradiotherapeutic option and should be investigated further with combinations of other chemotherapy regimens.

In recent years, however, some articles have shown that addition of induction chemotherapy before concurrent chemoradiotherapy adds toxicity and provides no survival benefit (24,25). In addition, National Comprehensive Cancer Network (NCCN) practice guideline recommends CDDP plus etoposide or vinblastin with concurrent radiotherapy as preferred standard of cares (category 2A) for patients with unresectable NSCLC (28). Further studies to investigate the role of induction chemotherapy followed by chemoradiotherapy may be not necessary until appearance of more active anticancer agents.

In conclusion, we failed to demonstrate promising efficacy of this regimen, and the development of a brand-new treatment strategy for combining chemotherapy with radiotherapy is necessary for the improvement of the prognosis of the patients with unresectable Stage III NSCLC.

Acknowledgements

We are indebted to Ms Mieko Imai and Ms Aya Kimura for data management, to Drs Naoki Ishizuka and Taro Shibata for statistical analysis and to Drs Kenjirou Kikuchi (Asahikawa Medical College) and Toshiaki Fujikane (National Dohoku Hospital) for their contributions in this study.

Funding

Supported in part by Grants-in-Aid for Cancer Research and for the Second-term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare (Tokyo).

Conflict of interest statement

None declared.

References

1. Bunn PA, Kelly K. New chemotherapeutic agents prolong survival and improve quality of life in non-small cell lung cancer: a review of the literature and future directions. *Clin Cancer Res* 1998;4:1087–100.
2. Non-small Cell Lung Cancer Collaborative Group. A meta-analysis using updated data on individual patients from 52 randomised clinical trials. *BMJ* 1995;311:899–909.
3. Dillman RO, Seagren SL, Propert KJ, Guerra J, Eaton WL, Perry MC, et al. A randomized trial of induction chemotherapy plus high-dose radiation versus radiation alone in stage III non-small-cell lung cancer. *N Engl J Med* 1990;323:940–5.
4. Le Chevalier T, Arriagada R, Quoix E, Ruffie P, Martin M, Tarayre M, et al. Radiotherapy alone versus combined chemotherapy and radiotherapy in nonresectable non-small-cell lung cancer: first analysis

of a randomized trial in 353 patients. *J Natl Cancer Inst* 1991;83: 417–23.
5. Sause WT, Scott C, Taylor S, Johnson D, Livingston R, Komaki R, et al. Radiation Therapy Oncology Group (RTOG) 88-08 and Eastern Cooperative Oncology Group (ECOG) 4588: preliminary results of a phase III trial in regionally advanced, unresectable non-small-cell lung cancer. *J Natl Cancer Inst* 1995;87:198–205.
6. Dillman RO, Herndon J, Seagren SL, Eaton WL, Jr, Green MR. Improved survival in stage III non-small-cell lung cancer: seven-year follow-up of Cancer and Leukemia Group B (CALGB) 8433 trial. *J Natl Cancer Inst* 1996;88:1210–5.
7. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985;260:14873–8.
8. Andoh T, Ishii K, Suzuki Y, Ikegami Y, Kusunoki Y, Takemoto Y, et al. Characterization of a mammalian mutant with a camptothecin-resistant DNA topoisomerase I. *Proc Natl Acad Sci USA* 1987;84: 5565–9.
9. Negoro S, Fukuoka M, Masuda N, Takada M, Kusunoki Y, Matsui K, et al. Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1991;83:1164–8.
10. Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, Nishiwaki Y, et al. A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *J Clin Oncol* 1992;10:16–20.
11. Ohe Y, Ohashi Y, Kubota K, Tamura T, Nakagawa K, Negoro S, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. *Ann Oncol* 2007;18:317–23.
12. Masuda N, Fukuoka M, Fujita A, Kurita Y, Tsuchiya S, Nagao K, et al. A phase II trial of combination of CPT-11 and cisplatin for advanced non-small-cell lung cancer. CPT-11 Lung Cancer Study Group. *Br J Cancer* 1998;78:251–6.
13. Schaake-Koning C, van den Bogaert W, Dalesio O, Festen J, Hoogenhout J, van Houtte P, et al. Effects of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. *N Engl J Med* 1992;326:524–30.
14. Hazuka MB, Crowley JJ, Bunn PA, Jr, O'Rourke M, Braun TJ, Livingston RB. Daily low-dose cisplatin plus concurrent high-dose thoracic irradiation in locally advanced unresectable non-small-cell lung cancer: results of a phase II Southwest Oncology Group study. *J Clin Oncol* 1994;12:1814–20.
15. Vokes EE, Weichselbaum RR. Concomitant chemoradiotherapy: rationale and clinical experience in patients with solid tumors. *J Clin Oncol* 1990;8:911–34.
16. Knox RJ, Friedlos F, Lydall DA, Roberts JJ. Mechanism of cytotoxicity of anticancer platinum drugs: evidence that cis-diamminedichloroplatinum (II) and cis-diammine-(1,1-cyclobutanedicarboxylato) platinum (II) differ only in the kinetics of their interaction with DNA. *Cancer Res* 1986;46:1972–9.
17. Kunitoh H, Watanabe K, Nagatomo A, Okamoto H, Kimbara K. Concurrent daily carboplatin and accelerated hyperfractionated thoracic radiotherapy in locally advanced nonsmall cell lung cancer. *Int J Radiat Oncol Biol Phys* 1997;37:103–9.
18. Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, et al. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *J Clin Oncol* 1989;7:1748–56.
19. World Health Organization. *WHO Handbook for Reporting Results of Cancer Treatment, Offset Publication No. 48*. Geneva: WHO 1979.
20. Tobinai K, Kohno A, Shimada Y, Watanabe T, Tamura T, Takeyama K, et al. Toxicity grading criteria of the Japan Clinical Oncology Group. The Clinical Trial Review Committee of the Japan Clinical Oncology Group. *Jpn J Clin Oncol* 1993;23:250–7.
21. Shimoyama M, Fukuda H, Saijo N, Yamaguchi N. Japan Clinical Oncology Group (JCOG). *Jpn J Clin Oncol* 1998;28:158–62.
22. Armitage P, Berry G. Survival analysis. *Statistical Methods in Medical Research*. 3rd ed. Oxford: Blackwell Scientific Publications 1994;469–92.
23. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;10:1–10.
24. Seulier JP, Lafitte JJ, Berghmans T, Van Houtte P, Lecomte J, Thiriaux J, et al. A phase III randomised study comparing two different dose-intensity regimens as induction chemotherapy followed by thoracic irradiation in patients with advanced locoregional non-small-cell lung cancer. *Ann Oncol* 2004;15:399–409.
25. Vokes EE, Herndon JE, 2nd, Kelley MJ, Cicchetti MG, Ramnath N, Neill H, et al. Induction chemotherapy followed by chemoradiotherapy compared with chemoradiotherapy alone for regionally advanced unresectable stage III non-small-cell lung cancer: Cancer and Leukemia Group B. *J Clin Oncol* 2007;25:1698–704.
26. Benson AB, 3rd, Ajani JA, Catalano RB, Engelking C, Kornblau SM, Martenson JA, Jr, et al. Recommended guidelines for the treatment of cancer treatment-induced diarrhea. *J Clin Oncol* 2004;22: 2918–26.
27. Furuse K, Fukuoka M, Kawahara M, Nishikawa H, Takada Y, Kudoh S, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. *J Clin Oncol* 1999;17:2692–9.
28. NCCN Clinical practice guidelines in Oncology: non-small cell lung cancer (V.2.2009). http://www.nccn.org.

British Journal of Cancer (2009) 101, 1549–1554

www.bjcancer.com

A phase-II trial of dose-dense chemotherapy in patients with disseminated thymoma: report of a Japan Clinical Oncology Group trial (JCOG 9605)

H Kunitoh[*,1,9], T Tamura[1], T Shibata[2], K Nakagawa[3], K Takeda[4], Y Nishiwaki[5], Y Osaki[6], K Noda[7], A Yokoyama[8], N Saijo[3,10] and JCOG Lung Cancer Study Group, Tokyo, Japan

[1]Department of Medical Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; [2]JCOG Data Center, Center for Cancer Control and Information Services, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; [3]Department of Medical Oncology, Kinki University School of Medicine, 377-2 Ohnohigashi, Osakasayama, Osaka 589-8511, Japan; [4]Department of Medical Oncology, Osaka City General Hospital, 2-13-22 Miyakojima-Hondori, Miyakojima-ku, Osaka 534-0021, Japan; [5]Department of Thoracic Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanohara, Kashiwashi, Chiba 277-8577, Japan; [6]Department of Internal Medicine, Asahikawa Medical College, 1-1-1 Higashinijou, Midorigaoka, Asahikawa, Hokkaido 078-8510, Japan; [7]Division of Thoracic Oncology, Kanagawa Cancer Center, 1-1-2 Nakao, Asahi-ku, Yokohama, Kanagawa 241-0815, Japan; [8]Department of Medical Oncology, Niigata Cancer Center, 2-15-3, Kawagishi-cho, Niigata-shi, Niigata 951-8566, Japan; [9]Department of Respiratory Medicine, Mitsui Memorial Hospital, 1 Kandaizumicho, Chiyoda-ku, Tokyo 101-8643, Japan; [10]National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwashi, Chiba 277-8577, Japan

BACKGROUND: To evaluate the safety and efficacy of dose-dense weekly chemotherapy in the treatment of advanced thymoma.

METHODS: Subjects comprised patients with histologically documented chemotherapy-naïve thymoma with stage-IVa or IVb disease. Thymic carcinoma, carcinoid or lymphoma cases were excluded. Patients received 9 weeks of chemotherapy: cisplatin (25 mg m^{-2}) on weeks 1–9; vincristine (1 mg m^{-2}) on weeks 1, 2, 4, 6 and 8; and doxorubicin (40 mg m^{-2}) and etoposide (80 mg m^{-2}) on days 1–3 of weeks 1, 3, 5, 7 and 9. Chemotherapy courses were supported by granulocyte colony-stimulating factor. Post-protocol local therapy was allowed.

RESULTS: From July 1997 to March 2004, 30 patients were entered. Three were ineligible due to different histology. Chemotherapy-associated toxicity was mainly haematological and was well tolerated, with no deaths due to toxicity, and 87% of patients completed the planned 9-week regimen. Overall response rate was 59%, with 16 of the 27 eligible patients achieving partial response. Median progression-fee survival (PFS) was 0.79 years (95% confidence interval: 0.52–1.40 years), and PFS at 1 and 2 years was 37 and 15%, respectively. Overall survival rates at 2 and 5 years were 89 and 65%, respectively.

CONCLUSION: In stage-IV thymoma patients, weekly dose-dense chemotherapy offers similar activity to conventional regimens.

British Journal of Cancer (2009) **101**, 1549–1554. doi:10.1038/sj.bjc.6605347 www.bjcancer.com
Published online 6 October 2009

Keywords: thymoma; chemotherapy; dose-dense; platinum; anthracycline; granulocyte colony-stimulating factor

Thymoma is a rare thoracic tumour, but remains one of the most common tumours originating in the mediastinum (Thomas *et al*, 1999; Giaccone, 2005; Girard *et al*, 2009). Clinical behaviour tends to be indolent, but dissemination into the pleural space eventually occurs and sometimes distant metastasis arise (Thomas *et al*, 1999). Thymoma is frequently associated with paraneoplastic syndromes such as myasthenia gravis or pure red cell aplasia (Thomas *et al*, 1999; Giaccone, 2005). No International Union Against Cancer (UICC) TNM classification is available, and the Masaoka classification has been widely used for clinical staging (Masaoka *et al*, 1981; Girard *et al*, 2009).

*Correspondence: Dr H Kunitoh; E-mail: hkkunito@mitsuihosp.or.jp
Presented in part at the 42nd Annual Meeting of the American Society of Clinical Oncology, June 2-June 6, 2006, Atlanta, GA, USA
Received 3 June 2009; revised 4 September 2009; accepted 4 September 2009; published online 6 October 2009

The majority of thymomas are discovered at a limited stage, representing Masaoka stage-I or II, and surgical resection is the treatment of choice for such cases (Thomas *et al*, 1999; Giaccone, 2005; Girard *et al*, 2009). Even when the tumour invades neighbouring organs, as stage-III disease, surgical resection with postoperative radiotherapy is the preferred treatment when complete resection can be achieved (Curran *et al*, 1988; Urgesi *et al*, 1990; Ogawa *et al*, 2002; Strobel *et al*, 2004).

Systemic chemotherapy is usually used for stage-IVa (with pleural or pericardial dissemination) or stage-IVb disease (with lymphogenous or haematogenous metastases), but optimal management is less well established (Thomas *et al*, 1999; Girard *et al*, 2009). Several reports have described favourable outcomes in limited numbers of patients with stage-IVa disease treated using multimodal treatment including surgery (Kim *et al*, 2004; Yokoi *et al*, 2007).

Conversely, thymomas are generally reported to be chemotherapy-sensitive tumours, with response rates of 50–70% to

combination chemotherapy (Fornasiero *et al*, 1990; Loehrer *et al*, 1994, 1997, 2001; Giaccone *et al*, 1996; Berruti *et al*, 1999; Kim *et al*, 2004; Lucchi *et al*, 2006; Yokoi *et al*, 2007). Active agents include cisplatin (CDDP), vincristine (VCR), doxorubicin (ADM), etoposide (ETP), cyclophosphamide (CPM) and ifosfamide (IFX). Recent reports have shown marginal activity of pemetrexed (Loehrer *et al*, 2006) and combined carboplatin and paclitaxel (Lemma *et al*, 2008).

Dose-dense chemotherapy with the CODE combination (CDDP–VCR–ADM–ETP) and addition of granulocyte colony-stimulating factor (G-CSF) can be safely administered to patients with advanced lung cancer (Murray *et al*, 1991; Fukuoka *et al*, 1997). Theoretically, this approach might be suitable for chemosensitive tumours such as small-cell lung cancer and thymoma (Goldie and Coldman, 1983, 1984; Levin and Hryniuk, 1987; Murray, 1987). Because some pilot data in Japan suggested that administration of 12 weeks of the CODE chemotherapy was barely feasible, subsequent Japanese trials used a modified schedule, which was shortened to 9 weeks (Fukuoka *et al*, 1997; Furuse *et al*, 1998).

In 1996, the Japan Clinical Oncology Group (JCOG) initiated two clinical trials for advanced thymoma: one aimed at evaluating the safety and efficacy of the CODE regimen in stage IV, disseminated thymoma (JCOG 9605), and the other aimed at evaluating the safety and efficacy of CODE combination chemotherapy followed by surgical resection and postoperative radiotherapy in initially unresectable stage-III thymoma (JCOG 9606). The primary endpoint in each study was progression-free survival (PFS). The results of JCOG 9605 are reported herein.

PATIENTS AND METHODS

Eligibility criteria

Patients with chemotherapy-naive, histologically documented thymoma at Masaoka stage IVa or IVb were eligible for entry into the study. Thymoma must have been confirmed histologically and thymic tumours with other histology, such as thymic carcinoma, carcinoid or lymphoma, were excluded. Each patient was required to fulfil the following criteria: age, 15–70 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS), 0–2; adequate organ function, that is, leukocyte count $\geqslant 4000\,\mu l^{-1}$, platelet count $\geqslant 10^5\,\mu l^{-1}$, hemoglobin $\geqslant 10.0\,g\,dl^{-1}$, serum creatinine $<1.5\,mg\,dl^{-1}$, creatinine clearance $\geqslant 60\,ml\,min^{-1}$, serum bilirubin $<1.5\,mg\,dl^{-1}$, serum alanine transaminase and aspartate transaminase levels less than double the upper limit of the institutional normal range; and PaO_2 $\geqslant 70\,mm\,Hg$. Exclusion criteria included uncontrolled heart disease, uncontrolled diabetes or hypertension, pulmonary fibrosis or active pneumonitis as evidenced on chest radiography, infections necessitating systemic use of antibiotics, disease necessitating emergency radiotherapy such as superior vena cava obstruction syndrome, active concomitant malignancy and women who were pregnant or lactating. Also excluded were those patients with grave complications of thymoma, such as pure red cell aplasia or hypogammaglobulinemia. Myasthenia gravis was allowed and these patients were not excluded *per se*.

Patient eligibility was confirmed by the JCOG Data Center before patient registration. This study protocol was approved by the institutional review board at each participating centre and written informed consent was obtained from all patients prior to enrolment.

Treatment Plan

Chemotherapy Patients received the 9-week CODE combination chemotherapy as described below. Each chemotherapeutic agent was administered intravenously.

Week 1: CDDP $25\,mg\,m^{-2}$ on day 1 with antiemetics and ample hydration; VCR $(1\,mg\,m^{-2})$ on day 1; ADM $(40\,mg\,m^{-2})$ on day 1 and ETP $(80\,mg\,m^{-2})$ on days 1–3.

Weeks 2, 4, 6 and 8: CDDP $(25\,mg\,m^{-2})$ on day 1 with antiemetics and ample hydration and VCR $(1\,mg\,m^{-2})$ on day 1.

Weeks 3, 5, 7 and 9: CDDP $(25\,mg\,m^{-2})$ on day 1 with antiemetics and ample hydration, ADM $(40\,mg\,m^{-2})$ on day 1 and ETP $(80\,mg\,m^{-2})$ on days 1–3.

Each week, G-CSF (filgrastim $(50\,\mu g\,m^{-2}\,day^{-1})$ or lenograstim $(2\,\mu g\,kg^{-1}\,day^{-1})$) was administered by subcutaneous injection, except on days when chemotherapy was administered or when leukocyte count was $\geqslant 10\,000\,\mu l^{-1}$. Corticosteroid was used only as part of the antiemetic regimen, and the specific drug and dosage were not regulated by the protocol.

Dose and schedule modifications were performed as follows: when leukocyte count decreased to $<2{,}000\,\mu l^{-1}$ or platelet count decreased to $<50\,000\,\mu l^{-1}$, chemotherapy was delayed by 1 week. If PS decreased to 3–4 or temperature reached $\geqslant 38.0°C$, therapy was likewise delayed for 1 week. No dose modification of chemotherapy drugs was adopted for toxicity.

Post-protocol therapy

Surgery or radiotherapy was allowed after the completion of chemotherapy, at the discretion of the attending physician, even in the absence of apparent tumour regrowth. Conversely, additional chemotherapy without evidence of disease progression was not allowed.

Post-treatment after disease progression was not limited by the study protocol.

Patient evaluation and follow-up

Before enrolment into the study, each patient underwent complete medical history taking and physical examination (including neurological check-up for signs of myasthenia gravis), determination of blood cell counts, serum biochemistry testing, arterial blood gas analysis, pulmonary function testing, electrocardiography, chest radiography, computed tomography (CT) of the chest, CT or ultrasonography of the upper abdomen, whole-brain CT or magnetic resonance imaging (MRI) and an isotope bone scan. Blood-cell counts, serum biochemistry testing and chest radiography were performed weekly during each course of chemotherapy.

The toxicity of chemotherapy was evaluated according to the JCOG Toxicity Criteria (Tobinai *et al*, 1993), modified from version 1 of the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Tumour responses were assessed radiographically according to the standard, two-dimensional WHO criteria (Miller *et al*, 1981), and were classified as complete response (CR), partial response (PR), no change (NC), progressive disease (PD) or non-evaluable (NE). After completion of the protocol therapy, patients were followed up with periodic re-evaluation, including chest CT every 6 months for the first 2 years and annually thereafter.

Central review

Radiographic reviews for the eligibility of enrolled patients and clinical responses were performed at the time of the study group meeting, held every 3–4 months. The study coordinator (H Kunitoh) and a few selected investigators from the group reviewed the radiographic films. The clinical response data presented below were all confirmed by this central review. Reviews of pathological specimens were not performed, because of insufficient logistics of the study group at the time of the study activation in 1997.

Endpoints and statistical considerations

The primary endpoint in each study was PFS. Due the rarity of the tumour and the accrual reported in US trials, which required 10 years to register 26 patients with locally advanced (stage-III) disease (Loehrer *et al*, 1997) and 9 years for 31 patients with disseminated (stage-IV) disease (Loehrer *et al*, 1994), we presumed we would be capable of accruing 30 patients in the target accrual period of 4 years. The sample size was, therefore, not determined based on statistical calculations. The expected PFS for the JCOG 9605 study was 2 years, which would give a 95% confidence interval of 1.3–3.0 years with 30 cases.

The initial study design thus envisioned enrolment of 30 fully eligible cases over 3 years for the study, with a follow-up period of 2 years.

Secondary endpoints included toxicity and safety, objective tumour response to chemotherapy, pattern of relapse, and overall survival (OS).

Progression-free survival and OS were calculated from the date of enrolment and estimated using the Kaplan–Meier method. Progression-free survival was censored at the last date verifiable as progression-free, and OS was censored as of the date of last follow-up. During the accrual period, an interim analysis for futility was planned after half of the patients had been registered and followed for $\geqslant 3$ months. All analyses were performed using SAS software version 8.2/9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

A total of 30 patients from seven institutions were enrolled from July 1997 to March 2004. Three patients were later found ineligible due to wrong histology, with two cases of thymic carcinoma and one case of carcinoid. These mistakes occurred due to technical problems in the patient registry. Since the ineligible cases did receive the protocol therapy, all 30 patients were analysed for characteristics and toxicity. Twenty-seven eligible patients were analysed for clinical response and survival (PFS and OS). Patient characteristics are shown in Table 1.

Table 1 Patient characteristics

Item	
Sex	
Male/female	16/14
Age (years)	
Median/range	47.5/29–69
ECOG performance status	
PS0/PS1/PS2	11/18/1
Masaoka stage	
IVa/IVb	22/8
Smoking history	
No	9
Yes (median pack-years)	21 (22)
Myasthenia gravis	
No/yes	28/2
Histology: thymoma and eligible	27
Lymphocyte predominance	12
Mixed cell	9
Epithelioid cell	4
Clear cell	1
Spindle cell	0
Unclassified	1
Histology: not thymoma (ineligible)	3
Carcinoma	2
Carcinoid	1
Lymphoma	0
Prior therapy	
None	26
Surgery	2
Surgery and radiation	2

Abbreviations: ECOG = Eastern Cooperative Oncology Group; PS = performance status.

Chemotherapy delivery and toxicity

Nine weeks of chemotherapy were performed for 26 of the original 30 patients (87%). The other four patients included one patient receiving 7 weeks, two receiving 6 weeks and one receiving 3 weeks of therapy. Median duration of chemotherapy for the 26 patients who underwent the planned nine cycles was 10 weeks (range, 9–12 weeks).

Table 2 summarises the major toxicities of chemotherapy, which were mainly haematological. Although 70% of patients experienced grade-IV neutropenia, this was generally transient and rarely complicated by infection/fever. Overall, toxicities were well tolerated and no deaths due to toxicity occurred.

Table 2 Toxicity of chemotherapy ($n = 30$)

Toxicity	Grades 1/2	Grade 3	Grade 4	%Grade 3/4
Leukopenia	3/6	12	8	67
Neutropenia	3/1	5	21	87
Anemia	0/5	25	ND	83
Thrombocytopenia	4/6	5	3	27
ALT	9/0	0	0	0
Creatinine	2/1	0	0	0
PaO_2	9/2	0	0	0
Emesis	13/11	2	ND	7
Diarrhoea	4/2	0	0	0
Stomatitis	4/3	0	0	0
Constipation	3/4	2	0	7
Neuropathy	11/2	0	ND	0
Infection	3/4	3	0	10

Abbreviations: ALT = alanine transaminase; ND = not defined (the JCOG toxicity criteria did not define grade IV in these toxicities).

Other and late complications

Four patients showed thymoma-related complications. One patient suffered from myasthenia gravis crisis occurring during chemotherapy, but subsequently recovered. Another patient showed newly diagnosed myasthenia gravis 2.5 years after completion of the protocol therapy, and thymectomy and resection of the residual tumour were performed. Two other cases had pure red cell aplasia occurring later in the clinical course with disease progression of the thymomas.

Clinical response to chemotherapy

Clinical responses of the 27 eligible patients to chemotherapy were judged radiologically and confirmed by central review. Responses were as follows: CR, 0 patients; PR, 16 patients; NC, 10 patients and PD, 1 patient. Overall response rate was 59% (95% confidence interval, 39–78%).

Post-protocol therapy

Post-protocol local therapy was administered to 18 of the 27 eligible patients (67%). Eight patients (all with stage-IVa disease) underwent surgical resection and 13 patients (nine with stage-IVa disease and four with stage-IVb disease) received thoracic radiotherapy, with three patients receiving both. Whether patients received local therapy after disease progression was not recorded on case report forms.

After disease progression, 16 of the 27 patients (59%) received additional chemotherapy. Post-protocol chemotherapy included platinum re-challenge, irinotecan, taxanes and investigational agents. Clinical response data to those therapies are not available.

PFS and OS

Survival data were finally updated in March 2006, 2 years after accrual of the last patient. Figure 1 shows PFS and OS curves of the 27 eligible patients. Median PFS was 0.79 years (95% confidence interval, 0.52–1.40 years) and PFS at 1 and 2 years was 37 and 15%, respectively. Median OS was 6.1 years and OS at 2 and 5 years was 89 and 65%, respectively.

Overall survival was longer for stage-IVa patients than for stage-IVb patients (Figure 2, median, 6.8 years and 3.5 years, respectively), but PFS was similar (Figure 3, median, 0.79 years for IVa patients and 0.78 years for IVb patients).

Pattern of relapse

As of the data cut-off, 26 of the 27 eligible patients had experienced tumour relapse. Sites of initial relapse comprised the primary site only in seven cases (27%), pleural or pericardial dissemination in seven cases (27%) and primary site and pleural/pericardial dissemination in nine cases (35%). Thus, 23 of the 26 patients with relapse initially showed regrowth of the primary and/or pleural or pericardial dissemination, with only three patients (12%) showing initial relapse at distant organs.

DISCUSSION

Few prospective trials of chemotherapy have been described for patients with advanced thymoma. Most prior studies have combined stage-III, localised disease and stage-IV, disseminated disease (Table 3). In addition, most have also included both thymoma and thymic carcinoma histology.

We have reported results for patients with stage-IV disease, for which systemic therapy should be the first choice. Among previous studies, only those from the ECOG separately reported results for stage-III and stage-IV patients (Loehrer *et al*, 1994, 1997). The ECOG took 9 years to accrue 31 patients with stage-IV disease, including patients with thymic carcinoma (Loehrer *et al*, 1994). We prospectively accrued patients with thymoma only and excluded thymic carcinoma, as thymoma and thymic carcinoma clearly differ in clinical presentation and prognosis, and trials involving these pathologies should, thus, be reported separately (Eng *et al*, 2004; Giaccone, 2005; Lemma *et al*, 2008).

Trials of systemic chemotherapy for thymoma have reported response rates of 50–90%, so this tumour is generally considered sensitive to chemotherapy (Thomas *et al*, 1999). Dose-dense chemotherapy such as the CODE four-drug combination has been argued to be theoretically suitable for the treatment of such chemosensitive tumours (Murray, 1987).

Although our results showed that dose-dense CODE chemotherapy could be safely administered to thymoma patients, efficacy was not remarkable. The overall response rate was about 60%, no different from prior reports employing conventional-dose chemotherapy (Table 3). Progression-free survival was 9 months, falling far short of the expected 2 years. Although OS studies compared favourably with the corresponding ECOG trial (Loehrer *et al*, 1994), attempting to reach a valid conclusion would be difficult due to the small sample sizes. In addition, OS could be

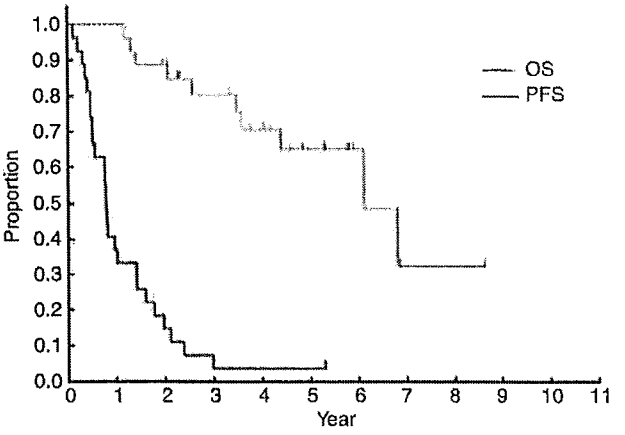

Figure 1 Progression-free survival and OS of the 27 eligible patients.

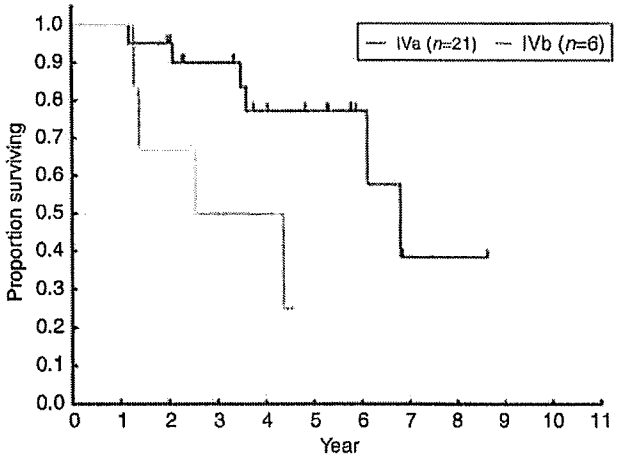

Figure 2 Overall survival according to Masaoka stage (stage IVa *vs* IVb).

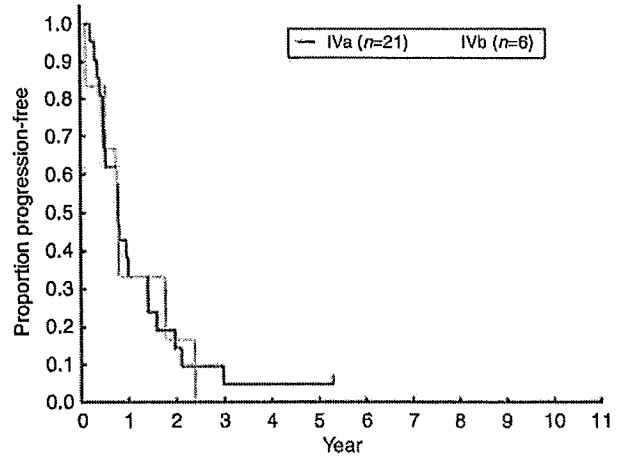

Figure 3 Progression-free survival according to Masaoka stage (stage IVa *vs* IVb).

Table 3 Reports of combination chemotherapy for thymoma

Regimen	Stage	Patients[a]	ORR	Reference
Anthracycline-containing regimens				
ADOC (S)	III/IV	32	91%	Fornasiero *et al* (1990)
PAC (G)	IV	30	50%	Loehrer *et al* (1994)
PAC (G)	III	23	70%	Loehrer *et al* (1997)
ADOC (S)	III/IV	16	81%	Berruti *et al* (1999)
PAC (G)	III/IV	22	77%	Kim *et al* (2004)
PAE (S)	III/IV	30	73%	Lucchi *et al* (2006)
CAMP (S)	III/IV	14	93%	Yokoi *et al* (2007)
CODE (G)	IV	27	59%	Current study
Non-anthracycline-containing regimens				
PE (G)	III/IV	16	56%	Giaccone *et al* (1996)
VIP (G)	III/IV	20	35%	Loehrer *et al* (1997)
CP (G)	III/IV	23	35%	Lemma *et al* (2008)

Abbreviations: ADOC = doxorubicin, cisplatin, vincristine, cyclophosphamide; CAMP = cisplatin, doxorubicin, methylpredonisolone; CODE = cisplatin, vincristine, doxorubicin, etoposide; CP = carboplatin, paclitaxel; G = prospective multicenter group trial; ORR = overall response rate; PAC = cisplatin, doxorubicin, cyclophosphamide; PAE = cisplatin, epidoxorubicin, etoposide; PE = cisplatin, etoposide; S = single-center experience; VIP = etoposide, ifosfamide, cisplatin. [a]Number of assessable patients.

greatly affected by post-study local therapy especially in patients with stage-IVa disease, as combined therapy trial including stage-IVa patients suggested (Kim *et al*, 2004). In fact, this might be one reason why OS of stage-IVa patients was much longer than that of stage-IVb patients, whereas PFS was similar.

It could be argued that shortened CODE chemotherapy, used in Japan due to feasibility problem, led to inadequate results due to insufficient total dosages of chemotherapy drugs. However, another intensive chemotherapy, ETP-IFX-CDDP (VIP) supported by G-CSF, has also reported disappointingly low response rates and no better survival (Loehrer *et al*, 2001). Hanna *et al* (2001) reported five patients with prior chemotherapy treated with high-dose chemotherapy and stem cell support, but concluded that no superiority to conventional therapy was evident. Taken together with our results, intensification of chemotherapy does not appear sufficiently promising for treating advanced thymoma.

Many prior chemotherapy studies have included platinum and anthracyclines in their regimens. Non-anthracycline approaches contained regimens such as VIP (Loehrer *et al*, 2001), ETP-CDDP (Giaccone *et al*, 1996) and paclitaxel-carboplatin (Lemma *et al*, 2008) tended to yield lower response rates of 32-56% as compared with regimens including anthracycline (Table 3). It might, thus, be suggested that both anthracycline and platinum should, thus, be included in thymoma chemotherapy, at least in current clinical practice.

Favourable results have recently been reported with multi-modality therapy, including surgical resection of stage-IVa disease (Kim *et al*, 2004; Yokoi *et al*, 2007). In fact, about two-thirds of eligible patients in our trial received local therapy after chemotherapy, including surgery in eight patients. This could have affected the outcome of the patients, as discussed above. However, small sample size and patient selection preclude reaching any definitive conclusion. When and what local therapy, if any, would benefit patients with disseminated thymoma, remains yet to be established. Further studies are warranted.

The present study shows several additional limitations. One is that we did not perform a central review of histology, and, thus, could not provide WHO classifications of histology (Okumura *et al*, 2002; Travis *et al*, 2004). This makes comparisons with results from other reports difficult. Central pathology review and preferably tissue collection would be very important in future trials.

In addition, due to the shorter-than-expected PFS, the planned CT scan interval of every 6 months might not have accurately evaluated PFS (Freidlin *et al*, 2007). Future trials might require more frequent scans.

In conclusion, we have reported that weekly dose-dense chemotherapy can be safely administered to patients with thymoma. However, efficacy seems similar to that in patients treated with conventional doses. More research on optimal systemic therapy and the role of local modalities would appear to be necessary.

ACKNOWLEDGEMENTS

This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan (11S-2, 11S-4, 14S-2, 14S-4, 17S-2, 17S-5).

REFERENCES

Berruti A, Borasio P, Gerbino A, Gorzegno G, Moschini T, Tampellini M, Ardissone F, Brizzi MP, Dolcetti A, Dogliotti L (1999) Primary chemotherapy with adriamycin, cisplatin, vincristine and cyclophosphamide in locally advanced thymomas: a single institution experience. *Br J Cancer* **81**: 841-845

Curran Jr WJ, Kornstein MJ, Brooks JJ, Turrisi 3rd AT (1988) Invasive thymoma: the role of mediastinal irradiation following complete or incomplete surgical resection. *J Clin Oncol* **6**: 1722-1727

Eng TY, Fuller CD, Jagirdar J, Bains Y, Thomas Jr CR (2004) Thymic carcinoma: state of the art review. *Int J Radiat Oncol Biol Phys* **59**: 654-664

Fornasiero A, Daniele O, Ghiotto C, Sartori F, Rea F, Piazza M, Fiore-Donati L, Morandi P, Aversa SM, Paccagnella A, Pappagallo GL, Fiorentino MV (1990) Chemotherapy of invasive thymoma. *J Clin Oncol* **8**: 1419-1423

Freidlin B, Korn EL, Hunsberger S, Gray R, Saxman S, Zujewski JA (2007) Proposal for the use of progression-free survival in unblinded randomized trials. *J Clin Oncol* **25**: 2122-2126

Fukuoka M, Masuda N, Negoro S, Matsui K, Yana T, Kudoh S, Kusunoki Y, Takada M, Kawahara M, Ogawara M, Kodama N, Kubota K, Furuse K (1997) CODE chemotherapy with and without granulocyte colony-stimulating factor in small-cell lung cancer. *Br J Cancer* **75**: 306-309

Furuse K, Fukuoka M, Nishiwaki Y, Kurita Y, Watanabe K, Noda K, Ariyoshi Y, Tamura T, Saijo N (1998) Phase III study of intensive weekly chemotherapy with recombinant human granulocyte colony-stimulating factor versus standard chemotherapy in extensive-disease small-cell lung cancer. *J Clin Oncol* **16**: 2126-2132

Giaccone G (2005) Treatment of malignant thymoma. *Curr Opin Oncol* **17**: 140-146

Giaccone G, Ardizzoni A, Kirkpatrick A, Clerico M, Sahmoud T, van Zandwijk N (1996) Cisplatin and etoposide combination chemotherapy for locally advanced or metastatic thymoma. A phase II study of the European Organization for Research and Treatment of Cancer Lung Cancer Cooperative Group. *J Clin Oncol* **14**: 814-820

Girard N, Mornex F, Van Houtte P, Cordier JF, van Schil P (2009) Thymoma: a focus on current therapeutic management. *J Thorac Oncol* **4**: 119-126

Goldie JH, Coldman AJ (1983) Quantitative model for multiple levels of drug resistance in clinical tumors. *Cancer Treat Rep* **67**: 923-931

Goldie JH, Coldman AJ (1984) The genetic origin of drug resistance in neoplasms: implications for systemic therapy. *Cancer Res* **44**: 3643-3653

Hanna N, Gharpure VS, Abonour R, Cornetta K, Loehrer Sr PJ (2001) High-dose carboplatin with etoposide in patients with recurrent thymoma: the Indiana University experience. *Bone Marrow Transplant* **28**: 435-438

Kim ES, Putnam JB, Komaki R, Walsh GL, Ro JY, Shin HJ, Truong M, Moon H, Swisher SG, Fossella FV, Khuri FR, Hong WK, Shin DM (2004) Phase II study of a multidisciplinary approach with induction chemotherapy, followed by surgical resection, radiation therapy, and consolidation chemotherapy for unresectable malignant thymomas: final report. *Lung Cancer* **44**: 369–379

Lemma GL, Loehrer Sr PJ, Lee JW, Langer CJ, Tester WJ, Johnson DH (2008) A phase II study of carboplatin plus paclitaxel in advanced thymoma or thymic carcinoma: E1C99. *J Clin Oncol* 26(15S): abstract 8018

Levin L, Hryniuk WM (1987) Dose intensity analysis of chemotherapy regimens in ovarian carcinoma. *J Clin Oncol* **5**: 756–767

Loehrer Sr PJ, Chen M, Kim K, Aisner SC, Einhorn LH, Livingston R, Johnson D (1997) Cisplatin, doxorubicin, and cyclophosphamide plus thoracic radiation therapy for limited-stage unresectable thymoma: an intergroup trial. *J Clin Oncol* **15**: 3093–3099

Loehrer Sr PJ, Jiroutek M, Aisner S, Aisner J, Green M, Thomas Jr CR, Livingston R, Johnson DH (2001) Combined etoposide, ifosfamide, and cisplatin in the treatment of patients with advanced thymoma and thymic carcinoma: an intergroup trial. *Cancer* **91**: 2010–2015

Loehrer Sr PJ, Kim K, Aisner SC, Livingston R, Einhorn LH, Johnson D, Blum R (1994) Cisplatin plus doxorubicin plus cyclophosphamide in metastatic or recurrent thymoma: final results of an intergroup trial. The Eastern Cooperative Oncology Group, Southwest Oncology Group, and Southeastern Cancer Study Group. *J Clin Oncol* **12**: 1164–1168

Loehrer Sr PJ, Yiannoutsos CT, Dropcho S, Burns M, Helft P, Chiorean EG, Nelson RP (2006) A phase II trial of pemetrexed in patients with recurrent thymoma or thymic carcinoma. *J Clin Oncol* 24(18S): abstract 7079

Lucchi M, Melfi F, Dini P, Basolo F, Viti A, Givigliano F, Angeletti CA, Mussi A (2006) Neoadjuvant chemotherapy for stage III and IVA thymomas: a single-institution experience with a long follow-up. *J Thorac Oncol* **1**: 308–313

Masaoka A, Monden Y, Nakahara K, Tanioka T (1981) Follow-up study of thymomas with special reference to their clinical stages. *Cancer* **48**: 2485–2492

Miller AB, Hoogstraten B, Staquet M, Winkler A (1981) Reporting results of cancer treatment. *Cancer* **47**: 207–214

Murray N (1987) The importance of dose and dose intensity in lung cancer chemotherapy. *Semin Oncol* **14**: 20–28

Murray N, Shah A, Osoba D, Page R, Karsai H, Grafton C, Goddard K, Fairey R, Voss N (1991) Intensive weekly chemotherapy for the treatment of extensive-stage small-cell lung cancer. *J Clin Oncol* **9**: 1632–1638

Ogawa K, Uno T, Toita T, Onishi H, Yoshida H, Kakinohana Y, Adachi G, Itami J, Ito H, Murayama S (2002) Postoperative radiotherapy for patients with completely resected thymoma: a multi-institutional, retrospective review of 103 patients. *Cancer* **94**: 1405–1413

Okumura M, Ohta M, Tateyama H, Nakagawa K, Matsumura A, Maeda H, Tada H, Eimoto T, Matsuda H, Masaoka A (2002) The World Health Organization histologic classification system reflects the oncologic behavior of thymoma: a clinical study of 273 patients. *Cancer* **94**: 624–632

Strobel P, Bauer A, Puppe B, Kraushaar T, Krein A, Toyka K, Gold R, Semik M, Kiefer R, Nix W, Schalke B, Muller-Hermelink HK, Marx A (2004) Tumor recurrence and survival in patients treated for thymomas and thymic squamous cell carcinomas: a retrospective analysis. *J Clin Oncol* **22**: 1501–1509

Thomas CR, Wright CD, Loehrer PJ (1999) Thymoma: state of the art. *J Clin Oncol* **17**: 2280–2289

Tobinai K, Kohno A, Shimada Y, Watanabe T, Tamura T, Takeyama K, Narabayashi M, Fukutomi T, Kondo H, Shimoyama M, Suemasu K (1993) Toxicity grading criteria of the Japan Clinical Oncology Group. The Clinical Trial Review Committee of the Japan Clinical Oncology Group. *Jpn J Clin Oncol* **23**: 250–257

Travis WB, Brambilla E, Muller-Hermelinck HK, Harris CC (2004) *Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart*. IARC Press: Lyon

Urgesi A, Monetti U, Rossi G, Ricardi U, Casadio C (1990) Role of radiation therapy in locally advanced thymoma. *Radiother Oncol* **19**: 273–280

Yokoi K, Matsuguma H, Nakahara R, Kondo T, Kamiyama Y, Mori K, Miyazawa N (2007) Multidisciplinary treatment for advanced invasive thymoma with cisplatin, doxorubicin, and methylprednisolone. *J Thorac Oncol* **2**: 73–78

Appendix 1

STUDY PARTICIPANTS

The following institutions and investigators participated in the trial:

Asahikawa Medical College (Yoshinobu Osaki), National Cancer Center Hospital East (Yutaka Nishiwaki, Kaoru Kubota, Nagahiro Saijo), National Cancer Center Hospital (Tomohide Tamura, Noboru Yamamoto, Hideo Kunitoh), Kanagawa Cancer Center (Kazumasa Noda, Fumihiro Oshita), Niigata Cancer Center Hospital (Akira Yokoyama, Yuko Tsukada), Kinki University Hospital (Kazuhiko Nakagawa, Isamu Okamoto) and Osaka City General Hospital (Koji Takeda, Haruko Daga).

Appendix 2

ACKNOWLEDGEMENTS

We thank Ms Mieko Imai for data management at the JCOG Data Center.

Drug Metab. Pharmacokinet. 24 (1): 118–126 (2009).

SNP Communication

Genetic Variations and Haplotype Structures of the Glutathione *S*-transferase Genes, *GSTT1* and *GSTM1*, in a Japanese Patient Population

Naoko TATEWAKI[1], Keiko MAEKAWA[1,2,*], Noriko KATORI[1,3], Kouichi KUROSE[1,4], Nahoko KANIWA[1,4], Noboru YAMAMOTO[5], Hideo KUNITOH[5], Yuichiro OHE[5], Hiroshi NOKIHARA[5], Ikuo SEKINE[5], Tomohide TAMURA[5], Teruhiko YOSHIDA[6], Nagahiro SAIJO[7], Yoshiro SAITO[1,2] and Jun-ichi SAWADA[1,2]

[1]*Project team for Pharmacogenetics, National Institute of Health Sciences, Tokyo, Japan*
[2]*Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, Tokyo, Japan*
[3]*Division of Drugs, National Institute of Health Sciences, Tokyo, Japan*
[4]*Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan*
[5]*Thoracic Oncology Division, National Cancer Center Hospital, National Cancer Center, Tokyo, Japan*
[6]*Genetics Division, National Cancer Center Research Institute, National Cancer Center, Tokyo, Japan*
[7]*Deputy Director, National Cancer Center Hospital East, Kashiwa, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Glutathione *S*-transferases (GSTs) play a vital role in phase II biotransformation of many synthetic chemicals including anticancer drugs. Deletion polymorphisms in *GSTT1* and *GSTM1* are reportedly associated, albeit controversial, with an increased risk in cancer as well as with altered responses to chemotherapeutic drugs. In this study, to elucidate the haplotype structures of *GSTT1* and *GSTM1*, genetic variations were identified in 194 Japanese cancer patients who received platinum-based chemotherapy. Homozygotes for deletion of *GSTT1* (*GSTT1*0/*0* or null) and *GSTM1* (*GSTM1*0/*0* or null) were found in 47.4% and 47.9% of the patients, respectively, while 23.2% of the patients had both *GSTT1* null and *GSTM1* null genotypes. From homozygous (+/+) and heterozygous (*0/+) patients bearing *GSTT1* and *GSTM1* genes, six single nucleotide polymorphisms (SNPs) for *GSTT1* and 23 SNPs for *GSTM1* were identified. A novel SNP in *GSTT1*, 226C>A (Arg76Ser), and the known SNP in *GSTM1*, 519C>G (Asn173Lys, **B*), were found at frequencies of 0.003 and 0.077, respectively. Using the detected variations, *GSTT1* and *GSTM1* haplotypes were identified/inferred. Three and six common haplotypes (N≥10) in *GSTT1* and *GSTM1*, respectively, accounted for most (>95%) inferred haplotypes. This information would be useful in pharmacogenomic studies of xenobiotics including anticancer drugs.

Keywords: *GSTT1*; *GSTM1*; nonsynonymous SNP; haplotype; haplotype-tagging SNP

Introduction

Glutathione *S*-transferases (GSTs) (EC 2.5.1.18) are dimeric phase II metabolic enzymes that mainly catalyze conjugation of reduced glutathione (GSH) with a variety of electrophilic compounds including carcinogens, therapeutic drugs and environmental toxins as well as endogenous substances.[1] In addition, GSTs possess selenium-independent GSH peroxidase activity to reduce organic hydroperoxides, and therefore, play significant roles in detoxification, occasionally toxification, and cellular protection against oxidative stress.[2] Noncatalytical-

Received; May 11, 2008, Accepted; August 20, 2008
**To whom correspondence should be addressed:* Keiko MAEKAWA, Ph.D., *Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel. +81-3-3700-9453, Fax. +81-3-5717-3832, E-mail: maekawa@nihs.go.jp*
On April 28th, 2008, the novel variations described in this paper were not found in the Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/) or SNP500Cancer Database (http://snp500cancer.nci.nih.gov/).
This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences and in part by the Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare.

ly, GSTs modulate signaling pathways by interacting with protein kinases[3] and by binding numerous ligands for nuclear hormone receptors.[4]

Human GSTs are composed of three main families: cytosolic, mitochondrial and microsomal (or membrane-bound). The cytosolic family, which is principally involved in biotransformation of toxic xenobiotics, contains at least 17 genes subdivided into seven separate classes designated alpha, mu, pi, sigma, theta, zeta, and omega.[5,6] Increasing numbers of GST genes are identified as polymorphic.

The θ-class enzyme GSTT1 and the μ-class enzyme GSTM1 exhibit gene deletion polymorphisms (*GSTT1*0* and *GSTM1*0*, respectively).[7] The null genotype of *GSTT1* (*GSTT1*0/*0*) is found in 15–40% of Caucasians and 50–60% of Asians.[7] On the other hand, about half of both Japanese and Caucasians and 30% of Africans are homozygous for the *GSTM1* deletion (*GSTM1*0/*0*).[7] In intact *GSTM1*, alleles **A* and **B* are used to discriminate the single nucleotide polymorphism (SNP) with amino acid substitution (thereafter, nonsynonymous SNP), 519C>G (Asn173Lys) in exon 7, in which both alleles encode proteins that are catalytically identical for the substrates, 1-chloro-2,4-dinitrobenzene (CDNB), *trans*-4-phenyl-3-buten-2-one (tPBO) and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP).[8] In addition, a tandem duplication in *GSTM1* associated with ultrarapid enzyme activity was observed in Saudi Arabians.[9] A gene-dose effect has been clearly established: that is, homozygously deleted (**0/*0*), heterozygously (**0/+*) and homozygously intact (*+/+*) *GST* genotypes correspond to non-, intermediate, and high conjugators, respectively.[10,11]

A large number of association studies on *GSTM1* and *GSTT1* null genotypes have been performed with inter-individual differences in susceptibility to environmental toxins, cancer and other diseases, and in the outcomes of anticancer treatments. Increased risk of lung, bladder, breast and colon cancers were observed in carriers of *GSTM1* or *GSTT1* null genotypes, while other studies have reported controversial findings.[5-7] As for response to anti-cancer drugs, pharmacodynamic correlations have been investigated, but the obtained results are inconsistent.[6] It should be pointed out that despite the possible gene-dose effect, most association studies were only focused on null genotypes of *GSTM1* and/or *GSTT1*. Therefore, in addition to nonconjugators, discrimination between high and intermediate conjugators would be valuable to evaluate the clinical relevance of these GST loci. Also, certain SNPs in the intact genes might affect either the expression of the gene or the activity of the encoded enzyme.

In this study, we first determined the deletion genotypes (**0/0*, **0/+*, and *+/+*) of *GSTM1* and *GSTT1* by conventional PCR and TaqMan real-time quantitative PCR for 194 Japanese cancer patients treated by platinum-based chemotherapy. Then, we resequenced the homozygous and heterozygous intact *GSTM1* and *GSTT1* genes. Lastly, linkage disequilibrium (LD) and haplotype analyses were performed using the detected SNPs.

Materials and Methods

Human genomic DNA samples: All 194 patients participating in this study were administered carboplatin or nedaplatin in combination with paclitaxel for treatment of various cancers (mainly non-small cell lung cancers) at the National Cancer Center. Genomic DNA was extracted from blood leukocytes from all subjects prior to the chemotherapy. The ethical review boards of the National Cancer Center and National Institute of Health Sciences approved this study. Written informed consent was obtained from all subjects.

Conventional PCR amplification of the *GSTT1* deletion junction: We used the genotyping assay described by Sprenger *et al.*,[10] in which 1460 (for **0* allele) and 466 bp (for exon 5 of the wild-type) PCR fragments were coamplified by multiplex PCR. PCR reactions were performed according to their method with minor modification.[10] Briefly, PCR mixtures contained 100 ng of genomic DNA, 0.2 μM each of the 4 primers reported previously, 0.2 mM each of four deoxynucleotide triphospates (dNTPs), and 0.75 units of HotStarTaq polymerase (Qiagen, Tokyo, Japan) in a 50 μl volume. The PCR conditions were 95°C for 15 min, followed by 30 cycles of 94°C for 30 sec, and 65°C for 1.5 min. PCR fragments were analyzed on 1% agarose gels with ethidium bromide in TAE buffer.

Conventional PCR amplification of *GSTM1*: We used the method of McLellan *et al.* (1997),[9] in which exons 3 to 5 of *GSTM1* were coamplified with β-globin as an internal standard by multiplex PCR. The PCR reactions were carried out according to their method[9] except that 100 ng of genomic DNA and 0.75 units of HotStarTaq polymerase (Qiagen) were used in a 50 μl total volume. The PCR conditions were 94°C for 15 min, followed by 30 cycles of 94°C for 48 sec, 62°C for 48 sec, and 72°C for 1.5 min, and then a final extension for 5 min at 72°C.

Quantitative real-time PCR for *GSTM1* and *GSTT1*: Quantitative real-time PCR using the TaqMan (5′-nuclease) assay system was carried out according to the method of Covault *et al.*,[12] in which the amounts of target *GSTM1* or *GSTT1* were quantified relative to those of the reference β-2-microglobulin (*B2M*) or cannabinoid receptor 1 (*CNR1*), respectively. Briefly, triplicate reactions were performed for 5 ng of genomic DNA used as a template in 1x TaqMan Universal PCR Master Mix with Amp Erase (50 μl) (Applied Biosystems, Foster City, CA, USA). The thermal cycling conditions were 50°C for 2 min and then 95°C for 10 min, followed by 40 cycles of

95°C for 20 sec and 60°C for 1 min with the 7500 Real-Time PCR System (Applied Biosystems).

***GSTT1* DNA sequencing:** The heterozygous and homozygous samples for *GSTT1* (*0/+ and +/+), the 5′-flanking region (up to 801 bp upstream from the translation start site), all 5 exons with their surrounding introns and the 3′-flanking region were amplified by PCR and directly sequenced. For the 1st round PCR, the reaction mixtures contained 25 ng of genomic DNA, 1.25 units of Ex-Taq (Takara Bio. Inc. Shiga, Japan), 0.2 mM dNTPs, and 0.2 μM primers listed in **Table 1**. The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min; and then a final extension for 7 min at 72°C. The regions from 5′-flanking to exon 1 and from exon 4 to 3′-flanking were amplified separately by the nested PCR with Ex-Taq (1.25 units) and the primer sets (0.2 μM) listed in "2nd round PCR" of **Table 1**. The 2nd round PCR conditions were the same as described in the 1st round PCR. The 2nd round PCR products and the 1st round PCR products for exons 2 and 3 were then treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and were directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) with the sequencing primers listed in **Table 1** (Sequencing column). Excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany). Eluates were analyzed on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). All novel SNPs were confirmed by repeated sequencing of the PCR products generated by new genomic DNA amplifications. The genomic and cDNA sequences of *GSTT1* obtained from GenBank (NT_011520.11 and NM_000853.1, respectively) were used as reference sequences.

***GSTM1* DNA sequencing:** For samples with *0/+ and +/+, genetic variations were identified by resequencing. Particular attention was paid to avoid amplification of sequences of other homologous *GSTMs* because exon 8 of *GSTM1* is 99% identical to that of *GSTM2*.[13] We confirmed that PCR fragments were not amplified from samples with *GSTM1*0/*0* genotypes to evaluate primer specificities. The entire *GSTM1* gene except for the region through exon 8 to the 3′-flanking region was amplified in the 1st round of PCR from 25 ng of genomic DNA utilizing 1.25 units of Ex-Taq with 0.2 μM of primers listed in **Table 2**. Next, three regions (from 5′-flanking to exon 3, from exon 4 to 5, and from exon 6 to 7), were separately amplified in the 2nd round PCR from the 1st round PCR product by Ex-Taq (0.625 units) with 0.2 μM primers listed in **Table 2**. The region from exon 8 to the 3′-flanking was separately amplified from 25 ng of genomic DNA using 0.625 units of Ex-Taq with 0.2 μM primers (listed in **Table 2**). All PCR conditions were the same as those described for *GSTT1*. PCR products were then directly sequenced with the primers listed in

Table 1. *GSTT1* primer sequences

	Amplified and sequenced region		Forward primer		Reverse primer		PCR product (bp)
			Sequence (5′ to 3′)	Position[a]	Sequences (5′ to 3′)	Position[a]	
1st round PCR	multiplex	5′-flanking (up to −1366) to exon 1	CACTCCCGCCCCAAATTAGGTT	3776166	ATGATCCCCACCCCTTTATTCG	3774444	1723
		Exon 4 to 3′-flanking region	ATCACAAGGTCAGGAGATTG	3767902	ACTCTTGGCAAACATCAGGG	3766589	1314
							1257
		Exon 2	ACATAATCTCTTCTGCAAACTG	3773267	TGTCTCAAGGATACTCTCACCA	3772011	
							2010
		Exon 3	GCAAATTGTCAGAAAGGTTAAAGA	3770734	CCCACCTCCTGATTAGCTTAGAAG	3768725	
2nd round PCR		5′-flanking (up to −801) to exon 1	TTTCAGTGGGATTCGTTTTAGA	3775601	CCCCGTGGTCTATTCCGTGA	3774478	1124
		Exon 4 to 3′-flanking region	CATCACTAATCATTAGGGAA	3767648	CTGGGAAGGGGGTTGTCTTT	3766628	1021
Sequencing		5′-flanking (up to −801)	TTTCAGTGGGATTCGTTTTAGA	3775601	GGCTCGCTCATTTCACTTAG	3775090	
		Exon 1	GGTGGGAAATTCTGACACAC	3775162	CCCCGTGGTCTATTCCGTGA	3774478	
		Exon 2[b]	AAGGGACAAGGTAGTCAGTC	3772758	AACTGGAATAGCAGGAAGGC	3772099	
		Exon 3[b]	AAAAAAGCGACTATGTATGAAAT	3770153	AGATAAAATGGATGAACACATGGT	3769662	
		Exon 4	CATCACTAATCATTAGGGAA	3767648	CAGACTGGGGATGGATGGTTGT	3767204	
		Exon 5 to 3′-flanking region	CATCCCCAGTCTGTACCCTTTTCC	3767216	CTGGGAAGGGGGTTGTCTTT	3766628	

[a]The nucleotide position of the 5′ end of each primer on NT_011520.11.
[b]For exons 2 and 3, the 1st round PCR product was directly sequenced.

Table 2. *GSTM1* primer sequences

	Amplified and sequenced region	Forward primer		Reverse primer		PCR product (bp)
		Sequence (5′ to 3′)	Position[a]	Sequences (5′ to 3′)	Position[a]	
1st round PCR	5′-flanking (up to −1309) to exon 7	CCACAAACAAGTTTATTGGGCG	6136872	GTACTAGACATCAATGTCACCGTT	6141347	4476
	Exon 8 to 3′-flanking region	ACAGTGAGATTTTGCTCAGGTATT	6142766	CTCAATTCTAGAAAAGAGCGAG	6145058	2293
2nd round PCR	5′-flanking (up to −650) to exon 3	GACCACATTTCCTTTACTCTGG	6137531	TAAGAATACTGTCACATGAACG	6139231	1701
	Exon 4 to 5	TCTGTGTCCACCTGCATTCGTTCA	6139192	CTGAACACAAACTTTACCATAC	6139883	692
	Exon 6 to 7	CTAATAAATGCTGATGTATCCAAT	6140410	CCTACTATTGCCAGCTCCATCTAT	6141315	906
Sequencing	5′-flanking (up to −650)	GTCCTTCCTATACCACTGACAC	6137567	AACCGAGCAGGGCTCAGAGTAT	6138145	
	Exon 1 to 2	CCCTGACTTCGCTCCCGGAAC	6137956	GGACACCCGTCCCAATTAGACA	6138764	
	Exon 3	TCTGCCCACTCACGCTAAGTTG	6138577	TAAGAATACTGTCACATGAACG	6139231	
	Exon 4 to 5	TCTGTGTCCACCTGCATTCGTTCA	6139192	CTGAACACAAACTTTACCATAC	6139883	
	Exon 6 to 7	CTAATAAATGCTGATGTATCCAAT	6140410	CCTACTATTGCCAGCTCCATCTAT	6141315	
	Exon 8[b]	GAACTTCTGTTTCCCACATGAG	6143164	GAGTAAAGATGGGAATAAACAG	6143735	
	3′-untranslated and flanking region[b]	TCGTTCCTTTCTCCTGTTTATT	6143701	CCTTGGGGTCCTATTCAATGAG	6144362	

[a]The nucleotide position of the 5′ end of each primer on NT_019273.18.
[b]For the region from exon 8 to 3′-flanking, the 1st round PCR product was directly sequenced.

"sequencing" of **Table 2** as described above for *GSTT1*. All novel SNPs were confirmed by repeated sequencing of PCR products that were newly generated by amplification of genomic DNA. The genomic and cDNA sequences of *GSTM1* obtained from GenBank (NT_019273.18 and NM_000561.2, respectively) were used as reference sequences.

Linkage Disequilibrium (LD) and haplotype analyses: Hardy-Weinberg equilibrium and LD analyses were performed by SNPAlyze ver 7.0 (Dynacom Co., Yokohama, Japan). Pairwise LD ($|D'|$ and r^2 values) between two variations was calculated using 102 subjects bearing one or two *GSTT1* genes and 101 subjects bearing one or two *GSTM1* genes. Some haplotypes were unambiguous from subjects with heterozygous **0* alleles. Diplotype configurations were inferred based on estimated haplotype frequencies using Expectation-Maximization algorithms by SNPAlyze software, which can handle multiallelic variations. Haplotypes containing SNPs without any amino acid change were designated as **1*, and nonsynonymous SNP-bearing haplotypes were numerically numbered. Subtypes were named in their frequency order by use of alphabetical small letters.

Results

Determination of deletion polymorphisms in *GSTM1* and *GSTT1*: Both conventional PCR[10] and TaqMan real-time PCR[12] were used to identify deletion of *GSTT1*. By conventional PCR, 92 out of 194 subjects (frequency = 0.474) were assigned as *GSTT1*0/*0*. For all 92 samples with *GSTT1*0/*0*, no significant fluorescence derived from *GSTT1* amplification was detected by TaqMan real-time PCR (mean cycle threshold, Ct, 37.6). Eighty-two (frequency = 0.423) and 20 (frequency = 0.103) subjects were identified as heterozygous (**0/+*) and homozygous (*+/+*) for intact *GSTT1* by conventional PCR, respectively. In the TaqMan real-time PCR, the mean ± SD of relative amounts of *GSTT1* was 1.0 ± 0.111, and 0.448 ± 0.058 for homozygous and heterozygous *GSTT1* carriers, respectively (the mean value for the 20 homozygotes was set as 1). Since the maximum relative amount of *GSTT1* was 1.214, no gene duplication could be inferred for *GSTT1*. The assigned genotypes were consistent between both methods, and their frequencies (**Table 3a**) were in Hardy-Weinberg equilibrium ($p = 0.785$ by Pearson's chi-square test).

As for *GSTM1*, conventional PCR[9] indicated that 93 out of 194 subjects had a homozygous deletion of *GSTM1* (**0/*0*), and that the remaining 101 subjects were either heterozygotes (**0/+*) or homozygotes (*+/+*) for intact *GSTM1*. By real-time PCR, Ct values of 93 samples with the null genotypes were greater than 36.5, which exceeded the sensitivity limits (Ct = 35) of the real-time PCR detection system, indicating that both methods gave consistent results for *GSTM1*0/*0*. As for the 101 subjects with intact *GSTM1* genes (either **0/+* or *+/+*), the distribution of relative amounts of *GSTM1* was clustered into two groups with 1.0 ± 0.083 (16 homozygotes), and 0.51 ± 0.048 (85 heterozygotes) when the mean value of the 16 homozygotes was set as 1. No individuals showed relative amounts more than 1.216, suggesting that the duplication in *GSTM1*[9] was not present in our population. Thus, the frequencies of *GSTM1*0/*0*, **0/+*, and *+/+*, were 0.479, 0.438, and 0.082, respectively (**Table 3a**), and in Hardy-Weinberg equilibrium ($p = 0.576$ by the Pearson's chi-square test).

Table 3b summarizes the results of the distribution of *GSTM1* and *GSTT1* deletions in our Japanese population.