Cooperative Group Research Endeavors in Small-Cell Lung Cancer: Current and Future Directions

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Abstract

The International Lung Cancer Congress (ILCC), now in its ninth year, is a key forum for representatives of cooperative groups in North America, Europe, and Japan to discuss ongoing and planned clinical trials in lung cancer. Many of the significant strides in lung cancer treatment often originate from investigations designed within the cooperative group system and were a feature of the 2008 ILCC. Small-cell lung cancer (SCLC) represents 15% of all lung cancers diagnosed annually and is characterized by rapid growth kinetics, disseminated metastases, and development of chemotherapy resistance. Many questions remain regarding the optimal use of radiation therapy and approaches for enhancing the effects of chemotherapy to improve clinical outcomes. Herein, we explore and outline the scientific vision of each cooperative group's SCLC research portfolio, as presented at the 2008 ILCC. Highlights include an ongoing Intergroup phase III study exploring differing radiation therapy schemes for limited-stage SCLC and a Southwest Oncology Group 0124 trial establishing platinum/etoposide as the standard of care for untreated extensive-stage SCLC in North America. Continued research efforts sponsored by these groups will represent the future of SCLC diagnosis and management.

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Introduction

Lung cancer is a strikingly prevalent malignancy and is the leading cause of cancer-related death in worldwide. Small-cell lung cancer (SCLC) represents 15% of all lung cancers, and in 2009, an estimated 32,000 new cases will be diagnosed in the United States.[1] Small-cell lung cancer is characterized by aggressive growth kinetics and disseminated metastases, with 60%-70% of patients presenting with advanced- (or "extensive-") stage disease. Despite high initial tumor response rates following platinum-based chemotherapy, SCLC rapidly develops drug resistance, subsequently leading to tumor progression and patient death. Unfortunately, progress in SCLC management has been agonizingly slow, with a glaring lack of therapeutic advances, despite a wealth of new chemotherapeutic drug classes and targeted agents. With median survivals of 7-11 months and a 2-year survival rate of < 5% for patients with extensive-stage disease, the need to improve outcomes is apparent.[2]

The US cooperative groups, sponsored by the taxpayer-supported National Cancer Institute, as well as cooperative groups from Canada, Europe, and Asia, all play a critical role in overcoming the slow progress in SCLC drug development by incorporating SCLC-specific clinical trials into their respective research portfolios. Within the United States, there are 4 general oncology cooperative groups active in lung cancer research: the Cancer and Leukemia Group B (CALGB), Eastern Cooperative Oncology Group (ECOG), North Central Cancer Treatment Group (NCCTG), and the Southwest Oncology Group (SWOG).[3] The CALGB, ECOG, and SWOG include member institutions from throughout the country, whereas NCCTG is a regional cooperative group centered at the Mayo Clinic. Within Canada, the National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) oversees cooperative oncology efforts. In addition, a focused cooperative oncology

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group that plays a pivotal role and crosses the US/Canadian border is the Radiation Therapy Oncology Group (RTOG). The 2008 International Lung Cancer Congress (ILCC), now in its ninth year, provides a unique forum to gather representatives from the North American cooperative groups as well as international groups such as the European Organization for Research and Treatment of Cancer (EORTC) and the Japan Clinical Oncology Group (JCOG). This article, the fourth in a series that outlines the scientific vision of each group, will focus on clinical research in SCLC.

To provide a foundation for discussion, one must first consider current treatment perspectives in SCLC. The standard therapeutic approach for patients with limited-stage SCLC (LS-SCLC) who are not candidates for a clinical protocol is 4 cycles of chemotherapy with concurrent thoracic irradiation. Based on its preclinical synergy and superiority in efficacy and tolerability with concomitant irradiation, cisplatin and etoposide chemotherapy has supplanted alkylator/anthracycline–based regimens as the chemotherapy backbone.[4] Thoracic irradiation results in local control and a survival benefit; however, the timing of radiation appears critical.[5,6] For example, early concurrent chemoradiation yields a small, but significant, survival advantage when compared with late concurrent or sequential thoracic irradiation; yet, the optimal radiation dose and fractionation regimen remains controversial.[7,8] For patients with excellent performance status and an adequate baseline pulmonary reserve, administration of twice-daily thoracic irradiation to 45 Gy with cisplatin/etoposide has shown encouraging long-term survival results.[9] However, in practice, this schedule is logistically difficult to administer and yet unknown to be superior to a biologically equivalent dose of a once-daily thoracic irradiation regimen. Patients with LS-SCLC who attain a complete response (CR) after concurrent chemoradiation are offered prophylactic cranial irradiation (PCI) based on a meta-analysis reporting a 5.4% improvement in 3-year overall survival (OS; 20.7% PCI-treated vs. 15.3% control) and a 25% reduction in the incidence of brain metastases (33.1% PCI-treated vs. 58.6% control).[10]

In North America and Europe, the cornerstone of treatment for extensive-stage SCLC (ES-SCLC) consists of platinum (cisplatin or carboplatin) and etoposide chemotherapy. The primary role of radiation therapy is for palliating symptomatic sites of disease. Recently, PCI has been incorporated into the treatment algorithm on the basis of results from a phase III clinical trial randomizing 286 patients with ES-SCLC with any response to initial chemotherapy to either PCI or observation.[11] At 1 year, PCI significantly reduced the incidence of symptomatic brain metastases (14.4% PCI-treated vs. 40.4% control; hazard ratio [HR], 0.27; $P < .001$) and increased OS (27.1% PCI-treated vs. 13.3% control; [HR], 0.68; $P = .003$). Indeed, this has led to the recommendation that PCI be offered for patients with ES-SCLC who respond to first-line chemotherapy, after a thorough discussion of the potential risks and benefits.

Unfortunately, the disease recurs in the majority of patients shortly after initial treatment. Although second-line chemotherapy can result in tumor regression, responses are short-lived, and median survival is often < 6 months.[2] A key factor guiding the selection of future therapy, and its possible efficacy, is the type of response gained after exposure to a first-line platinum-based regimen. Historically, patients are classified into 1 of 3 groups of relapsed disease: platinum sensitive, platinum resistant, or refractory. Platinum sensitivity is arbitrarily defined as a chemotherapy-free interval > 90 days, whereas patients with platinum-resistant disease have recurrent disease within 90 days of completing chemotherapy.[2] Refractory SCLC refers to those who do not respond to, or progress during, first-line chemotherapy. Patients with platinum-resistant and refractory disease are often grouped together and generally have poor responses to subsequent chemotherapy (≤ 10%) and shorter median survivals than patients with platinum-sensitive disease. Although there is no standard second-line treatment option, a number of agents have shown single-agent activity, such as the camptothecin analogues (topotecan, irinotecan), paclitaxel, vinorelbine, and gemcitabine.[2] Multiple-agent regimens, such as retreatment with platinum/etoposide, are also a common treatment choice for platinum-sensitive tumors. In the late 1990s, a randomized phase III trial for patients with recurrent SCLC compared single-agent topotecan with cyclophosphamide, doxorubicin, and vincristine (CAV) and found topotecan to be equally efficacious but with greater palliative effects on common lung cancer symptoms.[12] Topotecan, as a result of its US Food and Drug Administration (FDA) approval for second-line SCLC therapy in platinum-sensitive relapsed disease, has emerged as the standard of comparison in most phase III clinical trials.[13]

These perspectives highlight the current state of SCLC management, which has not changed significantly in the past decade. We will now explore the scientific progress and research endeavors pursued by the large multi-institutional cooperative groups.

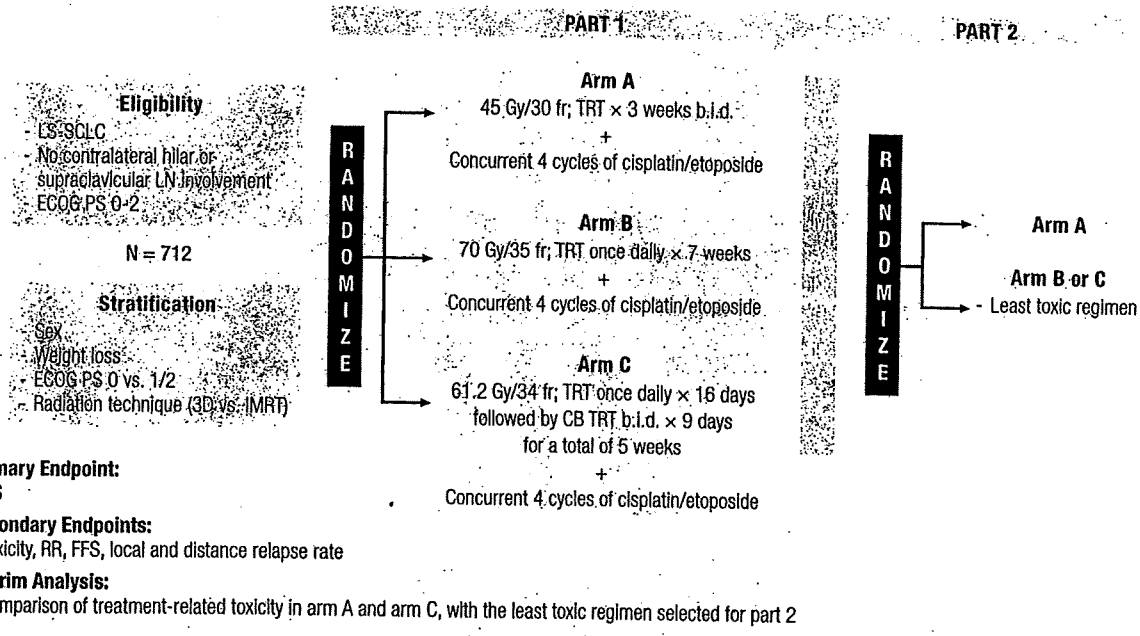
Cancer and Leukemia Group B

In 1987, the CALGB published a seminal report (CALGB 8083) describing the benefits of thoracic irradiation when given concurrently with chemotherapy for patients with LS-SCLC.[14] Improvements in local control, failure-free survival, and OS strengthened the case for shifting the standard of care to a chemoradiation therapy approach. Unfortunately, in 2009, many questions still remain unanswered regarding the optimal dose and delivery of thoracic irradiation.

Cancer and Leukemia Group B has been instrumental in exploring the 70-Gy maximum-tolerated dose (MTD) of once-daily radiation therapy in a phase II setting.[13,15] For example, CALGB conducted CALGB 39808, in which 57 patients with LS-SCLC were treated with 70 Gy in 35 once-daily fractions concurrently with carboplatin/etoposide following 2 cycles of induction paclitaxel and topotecan.[16] The reported 2-year survival was 48%, and the incidence of grade 3 dysphagia was 16%. However, the experience with 70 Gy of concurrent thoracic chemoradiation remains limited and, as a consequence, the de facto practice still calls for once-daily radiation therapy to be delivered at a total dose of 50-60 Gy in 1.8-2.0–Gy fractions.

Hyperfractionating radiation therapy is believed to offer additional clinical benefits. An Intergroup 0096 phase II trial randomized 417 patients to receive 4 cycles of cisplatin/etoposide with either 45 Gy of concurrent thoracic irradiation given twice daily over 3 weeks or once-daily for 5 weeks. Thoracic irradiation was scheduled to coincide with the start of chemotherapy. This pivotal trial found a significant 5-year OS benefit favoring twice-daily

Figure 1 **CALGB 30610/RTOG 0538 Treatment Schema: Phase III Trial Comparing Thoracic Radiation Therapy Regimens in Limited-Stage Small-Cell Lung Cancer**



Abbreviations: 3D = 3-dimensional conformal radiation therapy; b.i.d. = twice daily; CALGB = Cancer and Leukemia Group B; CB = concomitant boost; ECOG = Eastern Cooperative Oncology Group; FFS = failure-free survival; fr = fractions; IMRT = intensity-modulated radiation therapy; LN = lymph node; LS-SCLC = limited-stage small-cell lung cancer; OS = overall survival; PS = performance status; RR = response rate; RTOG = Radiation Therapy Oncology Group; TRT = thoracic radiation therapy

thoracic irradiation compared with once-daily fractionation (26% vs. 16%; $P = .04$) and a lower incidence of local failure (36% vs. 52%; $P = .06$).[9] Grade 3 esophagitis was the most significant toxicity with twice-daily radiation therapy (26% twice-daily vs. 11% once-daily), but the incidence of grade 4 esophagitis did not differ between regimens.

Radiation Therapy Oncology Group has examined an alternative fractionation scheme using a concomitant boost technique to escalate dose while keeping the total treatment duration at 5 weeks. Initially, thoracic irradiation is administered once-daily for 3 weeks, followed by 2 weeks of twice-daily thoracic irradiation. This dose/fractionation regimen is hypothesized to counteract accelerated repopulation, the increased tumor cell growth rate that is known to often occur several weeks into treatment. The MTD for the concomitant-boost technique, when combined with cisplatin/etoposide chemotherapy, has been determined at 61.2 Gy.[17] Thus, there are 3 plausible treatment regimens for delivering concurrent thoracic radiation therapy in LS-SCLC at relatively similar biologically effective doses: (1) CALGB's 70-Gy once-daily fractionation for 7 weeks, (2) the Intergroup 0096 regimen of 45-Gy twice-daily fractionation for 3 weeks, and (3) RTOG's 61.2-Gy concomitant-boost technique for 5 weeks duration.

To address the important radiation therapy questions of optimal dose and fractionation schemes, CALGB 30610, an Intergroup study, has now been developed (Figure 1). This pivotal phase III trial for patients with treatment-naive LS-SCLC is the first of its kind in well over a decade. It consists of 2 parts; part 1 has 3 treatment arms with patients randomized in a 1:2:2 fashion: arm A, 45 Gy (1.5 Gy twice daily × 3 weeks); arm B, 70 Gy (2.0 Gy once daily × 7 weeks); arm C, 61.2 Gy (1.8 Gy once daily × 16 days followed by 1.8 Gy twice daily × 9 days for a total duration of 5 weeks). Four cycles of cisplatin and etoposide are given concurrently, starting on day 1 of radiation therapy for all arms of this study. After interim analysis for toxicity assessment, only 1 experimental arm (arm B or arm C) will be selected for further accrual in part 2 of the study. The primary endpoint will be OS, and the projected total accrual is approximately 712 patients.

Several randomized trials have attempted to build on the platform of platinum/etoposide chemotherapy for ES-SCLC; however, these attempts have been met with disappointing results. For example, the addition of topotecan consolidation, paclitaxel, BEC2 vaccination, or thalidomide to the platinum/etoposide backbone have not shown any significant survival advantage.[18-22] Furthermore, CALGB 30103, a randomized phase II trial, evaluated the Bcl-2 antisense oligonucleotide, oblimersen (G3139), in combination with carboplatin/etoposide in 56 chemotherapy-naive patients with ES-SCLC. Although Bcl-2 is an overexpressed apoptotic inhibitor implicated in SCLC oncogenesis and chemotherapy resistance, CALGB 30103 suggested poorer clinical outcomes for patients who received oblimersen than for those who did not (1-year OS rates, 24% and 47%).[23]

Sunitinib, an oral small-molecule, multitargeted receptor tyrosine kinase inhibitor, has been FDA approved for the treatment of patients with renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumors. It has potent inhibitory effects of the platelet-derived growth factor receptors (PDGFRs)-α and

-β, vascular endothelial growth factor receptors (VEGFRs)-1, -2, and -3, stem cell factor receptor (KIT), Fms-like tyrosine kinase-3 (FLT-3), colony stimulating factor receptor (CSF)-1R, and the glial cell line–derived neurotrophic factor receptor (RET). Given its promiscuity in inhibition, sunitinib is hypothesized to affect multiple hallmarks of cancer, including angiogenesis and tumor cell proliferation. CALGB 30504 is an ongoing phase I/II clinical trial investigating the combination of sunitinib plus cisplatin/etoposide for patients with ES-SCLC. The phase I portion of the trial will determine the MTD to be used for the phase II portion. Sunitinib will be given daily concurrent with 6 cycles of cisplatin/etoposide, followed by maintenance sunitinib until the development of progressive disease (PD) or excessive toxicity. The phase II portion of the trial will randomize patients, after initial treatment with sunitinib plus cisplatin/etoposide, to maintenance therapy with either sunitinib or placebo. The primary endpoint will be progression-free survival (PFS), with an accrual goal of 107 patients.

Eastern Cooperative Oncology Group

Bevacizumab, a monoclonal antibody (MoAb) targeting VEGF, has shown to improve survival when combined with chemotherapy in patients with advanced NSCLC, as described in the ECOG 4599 trial.[24] Given these positive results, further evaluation of bevacizumab was felt to be warranted in SCLC because of its high degree of vascularization and VEGF expression.[25] ECOG 3501, a phase II trial of bevacizumab with cisplatin/etoposide in ES-SCLC, has completed accrual. A 21-day cycle of intravenous (I.V.) cisplatin 60 mg/m^2 day 1, etoposide 120 mg/m^2 days 1-3 I.V., and bevacizumab 15 mg/m^2 day 1 was administered for 4 cycles with maintenance bevacizumab given thereafter until PD or unacceptable toxicity. The primary endpoint was to detect an improvement in 6-month PFS from 16% to 33% in 66 patients. Updated survival analysis reported at the 2008 ILCC showed a 6-month PFS of 35% and a 1-year OS rate of 37%.[26] Median PFS and OS were 4.7 months and 11.1 months, respectively. Of the evaluable patients, there were no grade 3/4 hemorrhagic events, despite the known predisposition for SCLC to be centrally located. In another nonrandomized phase II study, CALGB 3036, 72 patients with previously untreated ES-SCLC received a maximum of 6 cycles of cisplatin 30 mg/m^2 days 1 and 8 I.V., irinotecan 65 mg/m^2 days 1 and 8 I.V., and bevacizumab 15 mg/m^2 day 1 without maintenance therapy. The regimen was feasible, and the 1-year PFS and OS rates were 18.3% and 48.9% (median PFS, 7.1 months; median OS, 11.7 months), respectively.[27] VEGF and PDGF levels showed no correlation with response, PFS, or OS. Overall, these studies are forming the rationale for the industry to evaluate bevacizumab in the phase III setting.

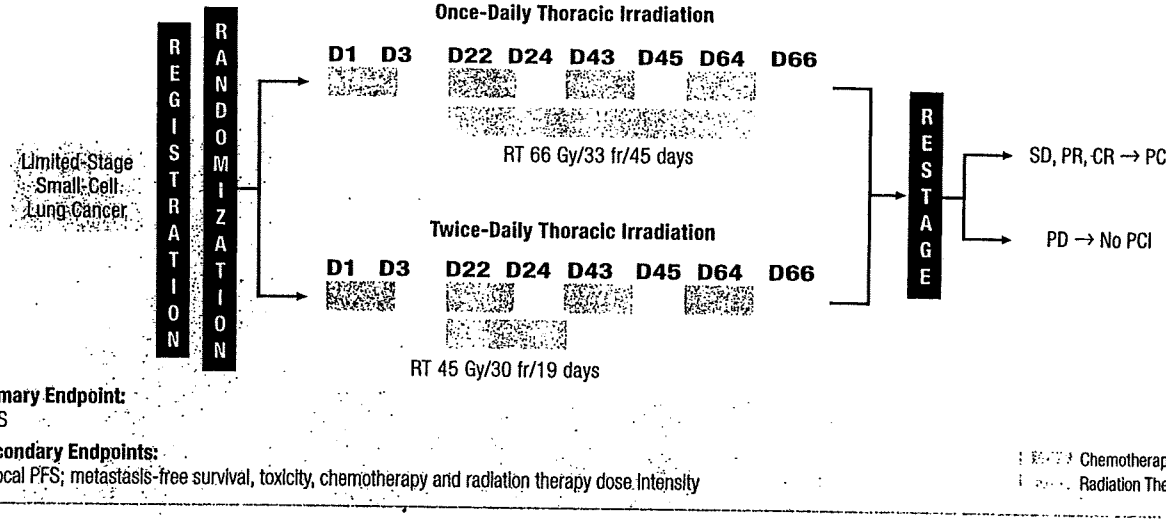
The Hedgehog (Hh) pathway is an essential embryonic signaling cascade implicated as an oncogenic catalyst in a variety of malignancies. There is evidence supporting persistent activation of the Hh pathway in SCLC, and in cell lines treated with a potent Hh inhibitor, cyclopamine, significant growth inhibition has been observed.[28,29] GDC-0449 is an orally bioavailable synthetic inhibitor of Hh signal transduction and has shown safety and clinical benefit in a phase I clinical trial for patients with advanced solid tumors.[30] Similarly, inhibition of the insulin-like growth factor (IGF) pathway is a promising new target with therapeutic efficacy in a variety of tumor models. This pathway is thought to mediate chemotherapy resistance as well as resistance to certain novel agents in SCLC.[31,32] Cixutumumab (IMC-A12), a MoAb targeting the IGF type 1 receptor (IGF-1R), is in clinical development. ECOG is proposing an ECOG 1508 three-armed, randomized phase II trial to determine "proof of activity." Patients with ES-SCLC will be randomized to receive (1) cisplatin/etoposide alone, (2) cisplatin/etoposide plus GDC-0449, or (3) cisplatin/etoposide plus cixutumumab for a total of four 21-day cycles. PFS is the planned primary endpoint, and the statistical design will include 74 patients per arm to have 85% power to detect a 33% reduction in the HR for PFS, corresponding to a 50% improvement in median PFS from 5.0 months to 7.5 months. Extensive correlative analysis will be integrated within this trial, with particular emphasis on Hh ligand and IGF-1R expression.

European Organization for the Research and Treatment of Cancer

The EORTC extends over multiple European countries and is a key contributor to clinical lung cancer research. Building upon the Intergroup 0096 study in LS-SCLC, the CONVERT (Concurrent ONce-daily VErsus Radiotherapy Twice-daily) trial hypothesizes that increasing the total dose of once-daily thoracic irradiation will improve efficacy and negate the benefit of twice-daily fractionation, thus making the once-daily regimen more practical and logistically easier to deliver. The CONVERT trial is a 2-arm, multicenter, randomized phase III Intergroup trial comparing a once-daily with a twice-daily schedule, given concurrently with cisplatin and etoposide (Figure 2). The radiation therapy regimen put forth by the Intergroup 0096 trial (45 Gy, twice-daily fractionation over 3 weeks) will be compared with 66 Gy, once-daily fractionation over 6.5 weeks. Unlike in the CALGB 30610 trial, thoracic irradiation will commence with the second cycle of chemotherapy. The primary endpoint will be OS, and the goal for accrual is 532 patients within a 4-year time span. The study is currently open in a number of EORTC member institutions.

Amrubicin is a novel cytotoxic agent being evaluated for the treatment of patients with ES-SCLC. It is a completely synthetic 9-amino-anthracycline that is converted to its ^{13}C alcohol metabolite amrubicinol, which has greater antitumor activity than its parent molecule, in stark contrast to the traditional anthracycline derivatives, doxorubicinol and daunorubicinol.[31] Moreover, amrubicin has been found to be less cardiotoxic than doxorubicin in animal models.[33] In a study of patients with refractory and sensitive relapsed SCLC, amrubicin has shown activity as a single agent. The overall response rate (ORR) was approximately 50% in each group, and the median PFS, median OS, and 1-year survival times in the refractory and sensitive groups were 2.6 months and 4.4 months, 10.3 months and 11.6 months, and 40% and 46%, respectively.[34] EORTC 08062 is a phase II trial equally randomizing chemotherapy-naive patients with ES-SCLC to 1 of 3 treatment arms: arm 1, amrubicin 45 mg/m^2 on days 1-3; arm 2, amrubicin 40 mg/m^2 on days 1-3 plus cisplatin 60 mg/m^2 on day 1; and arm 3, cisplatin 75

Figure 2 Treatment Schema: Phase III CONVERT Trial



[a]Maximum of 6 cycles of cisplatin/etoposide.
Abbreviations: CONVERT = Concurrent ONce-daily VErsus Radiotherapy Twice-daily; CR = complete response; D = day; fr = fractions; OS = overall survival; PCI = prophylactic cranial irradiation; PD = progressive disease; PFS = progression-free survival; PR = partial response; RT = radiation therapy; SD = stable disease

Table 1 Japan Clinical Oncology Group Research Portfolio of Ongoing and Proposed Clinical Trials in Small-Cell Lung Cancer

Protocol Number	Phase	Population	Reference Arm	Experimental Arm	Accrual Target, N	Primary Endpoint
JCOG 0202	III	Treatment-naive LS-SCLC	Cisplatin/etoposide + RT → Cisplatin/etoposide	Cisplatin/etoposide + RT → Cisplatin/irinotecan	250	Overall survival
JCOG 0509	III	Treatment-naive ES-SCLC	Cisplatin/irinotecan	Cisplatin/amrubicin	282	Overall survival
JCOG 0605	III	Relapsed SCLC: sensitive	Nogitecan	Cisplatin/etoposide/irinotecan	180	Overall survival
[a]PC 705	II	Relapsed SCLC: refractory	–	Amrubicin	80	Response rate

[a]Proposed clinical trial in development.
Abbreviations: ES-SCLC = extensive-stage small-cell lung cancer; JCOG = Japan Clinical Oncology Group; LS-SCLC = limited-stage small-cell lung cancer; PC = protocol concept; RT = radiation therapy; SCLC = small-cell lung cancer

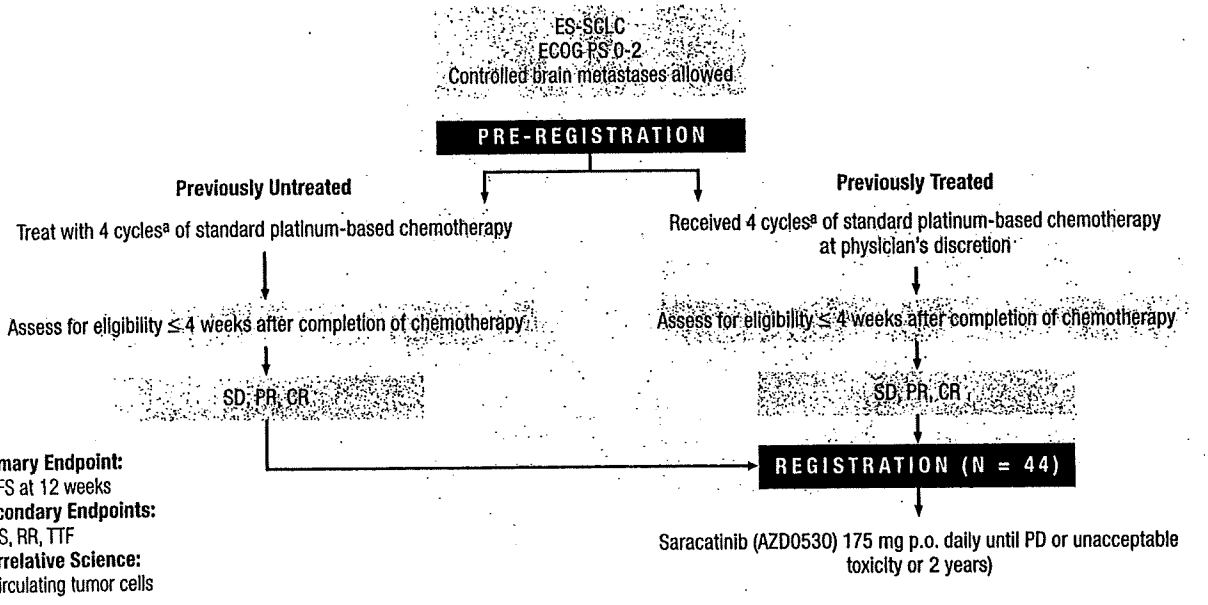
mg/m^2 on day 1 plus etoposide 100 mg/m^2 I.V. on day 1 followed by oral etoposide 200 mg/m^2 on days 2 and 3. In all arms, treatment is repeated every 21 days in the absence of progressive disease or unacceptable toxicity. Patients are stratified based on institution, sex, and performance status. The primary endpoint is RR, with secondary endpoints examining PFS, OS, and toxicity. Amrubicin is already approved in Japan and is currently being investigated in the United States in a multinational, randomized phase III trial for patients with SCLC who do not respond to first-line therapy. Considerable hope exists for this agent, but its role will need to be more clearly defined.

Finally, a proposal is in place for a phase II EORTC 08061 trial treating patients with chemotherapy-naive or sensitive relapsed ES-SCLC. Sunitinib will be given as a single oral agent (150-mg loading dose followed by 37.5 mg daily) until progressive disease. Disease control rate at 4 weeks after the start of treatment will be the primary endpoint.

Japan Clinical Oncology Group

Although there are a number of cooperative oncology groups in Japan, JCOG and the North Japan Lung Cancer Study Group (NJLCSG) are particularly active in SCLC research efforts. JCOG draws from its 190 participating institutions to enroll patients into its trials. In SCLC, there are 3 ongoing phase III trials, in addition to 1 phase II protocol in development that is evaluating amrubicin in the relapsed/refractory setting (Table 1). However, the featured trial at the 2008 ILCC was NJLCSG 0402, a randomized phase II trial comparing amrubicin with topotecan in previously treated SCLC. Sixty patients, stratified according to performance status and type of relapse (chemotherapy sensitive or refractory), were randomly assigned to receive amrubicin 40 mg/m^2 days 1-3 or topotecan 1 mg/m^2 days 1-5 for a minimum of three 21-day cycles. The primary endpoint of ORR was 38% for the amrubicin arm and 21% in the topotecan arm.[35] In sensitive relapse, the ORRs for amrubicin and topotecan were 53% and 21%, and in refractory relapse, 17% and 0%, respectively. There were no significant advantages of either therapy in median PFS and OS. Neutropenia was severe for those treated with amrubicin, with 79% of the patients experiencing grade 4 neutropenia and 14% of the patients experiencing febrile neutropenia. Moreover, 1 treatment-related death was observed resulting from sepsis. Encouragingly, amrubicin has activity, particularly in chemotherapy-refractory relapse, which is

Figure 3 NCCTG 0621 Treatment Schema: Phase II Trial of Saracatinib (AZD0530) in Extensive-Stage Small-Cell Lung Cancer



[a]1 cycle = 21 days.
Abbreviations: CR = complete response; ECOG = Eastern Cooperative Oncology Group; ES-SCLC = extensive-stage small-cell lung cancer; NCCTG = North Central Cancer Treatment Group; OS = overall survival; PD = progressive disease; PFS = progression-free survival; p.o. = orally; PS = performance status; RR = response rate; SD = stable disease; TTF = time to treatment failure

notoriously difficult to treat. Results are limited by the small sample size but still warrant further evaluation in larger-scale trials.

North Central Cancer Treatment Group

The NCCTG is a regional cooperative network based in the Mayo Clinic in Minnesota with a number of centers scattered across the United States, Canada, and Puerto Rico. The NCCTG customarily focuses on phase II clinical trial designs with novel therapeutic agents and also participates in Intergroup protocols such as the ongoing CALGB 30610 trial described earlier. The NCCTG research portfolio recently featured a phase II NCCTG 0621 trial evaluating a novel oral c-SRC inhibitor, saracatinib (AZD0530), administered daily in nonprogressing patients with ES-SCLC who received a maximum of 4 cycles of standard platinum-based chemotherapy (Figure 3). The trial was designed for a primary endpoint of 12-week PFS, and secondary endpoints included RR, OS, and time to treatment failure. Incorporated within the study is an intriguing analysis of the effects of saracatinib treatment on the levels of circulating tumor cells (CTCs) as well as correlative science attempting to determine potential predictive markers of response in CTCs. Complete analysis of the results are eagerly anticipated.

National Cancer Institute of Canada Clinical Trials Group

The NCIC-CTG is the only adult cooperative oncology group based in Canada with a national membership supporting a spectrum of clinical trials ranging from phase I testing of novel therapeutic agents to the conduct of large, randomized, controlled phase III trials. The importance of its contributions to the treatment of lung cancer is well recognized. Historically, the NCIC-CTG has been an active participant of SCLC trials initiated by other cooperative groups. NCIC-CTG BR.28, also known as the previously described CONVERT trial, is one such effort that has recently opened to accrual in NCIC-CTG member institutions.

Radiation Therapy Oncology Group

In lung cancer, RTOG research endeavors are intended to decipher the optimal methods of using radiation therapy in a consistently effective and safe manner. Besides being a key collaborator in the CALGB 30610 trial, designated as RTOG 0538 within the group, RTOG has been instrumental in discerning the best method of delivering PCI in LS-SCLC. RTOG 0212, closed to accrual in February 2008, was designed to determine the optimal dose of PCI after a meta-analysis suggested a reduced incidence of brain metastases with higher PCI doses. Patients with LS-SCLC who were complete responders to primary treatment were randomized to receive standard (25-Gy/10-fraction/12 days) or higher PCI doses (36-Gy) administered using either conventional (18 fractions/24 days) or accelerated hyperfractionated radiation therapy (24 twice-daily fractions/16 days). This phase II/III trial had significant contributions from CALBG, ECOG, EORTC, and SWOG, with results presented at the 2008 American Society of Clinical Oncology meeting. A total of 720 patients were enrolled, and although there was a nonsignificant trend for reduced 2-year brain metastases incidence with high-dose PCI compared with standard-dose PCI (24% vs. 30%; $P = .13$), there was a significantly marked increase in chest relapse (48% vs. 40%; $P = .02$) and mortality (2-year OS 37% with high-dose PCI vs. 42% with standard-dose PCI; $P = .03$).[36] Thus, the prevailing PCI dose of 25 Gy remains the standard of care for LS-SCLC.

Intergroup 0096 showed a survival benefit using an accelerated fractionation schedule compared with daily radiation therapy. RTOG 0239, a phase II trial, evaluated an innovative radiation therapy design where once-daily radiation therapy along with concurrent chemotherapy was given followed by a hyperfractionated schedule, a concomitant boost, in LS-SCLC (61.2 Gy/34 fractions). This schedule was found to be tolerable but was associated with a high incidence of myelosuppression.[37] RTOG 0623 is a phase II trial designed to overcome this adverse event by incorporating filgrastim with concurrent chemoradiation therapy and pegfilgrastim, with adjuvant cisplatin/etoposide chemotherapy in patients with LS-SCLC. Historically, hematopoietic growth factors have not been recommended during combined modality chemoradiation therapy based on early theoretical concerns that growth factors might release progenitor cells and expose them to the damaging effects of radiation therapy, but significant improvements in supportive care and delivery of radiation therapy could make these concerns less applicable. The primary endpoint of RTOG 0623 is to evaluate the safety and efficacy of filgrastim in reducing grade ≥ 3 neutropenia when given with concurrent chemoradiation. Unfortunately, this trial is accruing poorly and is expected to close soon.

Southwest Oncology Group

The premier effort of the SWOG research portfolio in SCLC is the recently reported S0124 phase III trial, a study in which CALGB, ECOG, and NCCTG also participated as part of the Intergroup.[38] This protocol duplicated the treatment regimen of a small phase III study conducted by JCOG (JCOG 9511) demonstrating the superiority of the cisplatin/irinotecan combination over cisplatin/etoposide in patients with chemotherapy-naive ES-SCLC with respect to RR, PFS, and OS.[39] After an interim analysis, the trial was closed to further accrual, with only 154 patients entered. Because of its small sample size and possible effects from pharmacogenomic differences between Japanese and North American populations, further confirmatory studies were prompted.

In a comparative North American and Australian phase III trial directed by the Hoosier Oncology Group, 331 patients were randomized to receive a modified dose schedule of cisplatin/irinotecan or cisplatin/etoposide.[40] The modified treatment regimens were intended to improve delivery, reduce toxicity, and be more consistent with the dosages and schedules administered in the United States.[31] In this trial, there were no differences in outcome between cisplatin/irinotecan and cisplatin/etoposide. Because of the differing dose schedules, questions remained regarding the validity of cisplatin/irinotecan as an optimal regimen for ES-SCLC.

The Southwest Oncology Group sought to conduct a confirmatory, appropriately powered trial (S0124) by designing a similar study to JCOG 9511 by using identical cisplatin/irinotecan and cisplatin/etoposide treatment doses and schedules, thereby determining whether the results were reproducible and relevant to a Western population.[38] Correlative studies were incorporated to seek out the possible role of population-related pharmacogenomic variability in irinotecan metabolism due to genetic polymorphisms. Over a 4-year time span, 671 patients were randomized to receive a maximum of 4 cycles of either cisplatin 60 mg/m^2 on day 1 plus irinotecan 60 mg/m^2 on days 1, 8, and 15 every 28-days or cisplatin 30 mg/m^2 on day 1 plus etoposide 100 mg/m^2 on days 1-3 every 21-days. Patients were stratified based on performance status, number of metastatic sites, weight loss, and lactate dehydrogenase levels. The primary endpoint was OS. Cisplatin/irinotecan efficacy outcomes were similar to cisplatin/etoposide, with an ORR of 60% versus 57%, median PFS of 5.8 months versus 5.2 months (P = .07), and a median OS of 9.9 months versus 9.1 months (P = .71), respectively.[38]

Evaluation of the adverse events between the S0124 and JCOG9511 trials demonstrated a significantly higher hematologic toxicity in Japanese patients compared with North American patients with either treatment regimen ($P \leq .02$), but the incidence of nonhematologic toxicities did not differ significantly. Of those enrolled in the S0124 trial, 142 patient samples were analyzed for pharmacogenetic variability of select genes in irinotecan metabolism performed on genomic DNA from peripheral blood mononuclear cells. Intriguingly, significant correlations for genetic polymorphisms and hematologic and gastrointestinal toxicities were found.[38]

Thus, S0124 did not confirm the results of JCOG9511 in a Western population. The putative mechanisms underlying the differences in efficacy and toxicity are hypothesized to be related to allelic variants of genes involved in irinotecan metabolism. SWOG has confirmed that in North America, platinum/etoposide remains the standard of care for previously untreated ES-SCLC.

The Southwest Oncology Group also recently reported S0435, a phase II study investigating the role of sorafenib in ES-SCLC.[41] Sorafenib, an oral multikinase inhibitor with effects on tumor proliferation and angiogenesis, is FDA-approved for the treatment of advanced renal cell and hepatocellular carcinoma. Patients with ES-SCLC treated with only 1 previous platinum-based chemotherapy regimen were stratified according to platinum sensitivity and treated with sorafenib 400 mg orally twice daily on a continuous basis for a 28-day cycle. Of 80 evaluable patients, 3 patients with platinum-sensitive disease had a partial response (PR; 8%), whereas only 1 patient with platinum-resistant disease had a PR (2%). The stable-disease rates were similar between both groups (32% and 31%, respectively). Median PFS was 2 months for both strata, and OS was 7 months for platinum-sensitive patients and 5 months for platinum-resistant patients. Given these results and the general tolerability of sorafenib, further study of this agent in SCLC is warranted.

Conclusion

Through their capacity to offer a wide range of scientific and patient resources, multi-institutional cooperative groups have a vital responsibility to ensure that significant strides in SCLC research continue to be made. As many SCLC trials have traditionally been underpowered, the importance of large collaborative research efforts to maximize accrual cannot be overemphasized. In addition, the trend to incorporate translational science studies into each trial offers an avenue to discern the underlying mechanisms of SCLC chemotherapy resistance and to perhaps develop future prognostic and predictive biomarker profiles. However, considerable work remains in order to overcome 2 decades of stagnant gains in SCLC management. The focus has shifted to first optimizing the delivery

of known effective treatments, such as thoracic irradiation in LS-SCLC, before expanding upon the paradigm so that therapeutic advances are built on a solid foundation. Moreover, novel targeted agents will certainly be added to the SCLC treatment armamentarium, ideally based on strong preclinical rationale and an appropriate "druggable" target, but to date, no targeted therapy has been approved for patients with SCLC. Indeed, the ongoing and planned research endeavors of the cooperative group system are essential to ensure that the future progress for SCLC management remains encouraging.

Disclosures

Dr. 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; has served as a paid consultant or been on an Advisory Board for AstraZeneca, Bayer Pharmaceuticals Corporation, Genentech, Inc., Pfizer Inc., Response Genetics, Inc., and sanofi-aventis U.S.; and is a member of the Speaker's Bureau for Eli Lilly and Company.

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SNP Communication

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

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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-

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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/).
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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
		Exon 2	ACATAATCTCTTCTGCAAACTG	3773267	TGTCTCAAGGATACTCTCACCA	3772011	1257
		Exon 3	GCAAATTGTCAGAAAGGTTAAAGA	3770734	CCCACCTCCTGATTAGCTTAGAAG	3768725	2010
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]	AAAAAAAGCGACTATGTATGAAAT	3770153	AGATAAAATGGATGAACAGATGGT	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.

About one-fourth (45 of 194 subjects) were null for both *GSTM1* and *GSTT1* genes.

Variations found in the intact *GSTT1* gene and their LD profiles: Six variations including three novel ones were found by sequencing the 5′-flanking regions, all 5 exons and their flanking regions in the 102 Japanese subjects with **0/+* and *+/+* genotypes (**Table 4**). All detected variations were in Hardy-Weinberg equilibrium ($p \geq 0.44$ by the χ^2 test or Fisher's exact test) when assuming the presence of three alleles (wild, variant and **0* alleles) at each site. One novel nonsynonymous variation, 226C>A (Arg76Ser), was heterozygous in one subject with two intact *GSTT1* genes, and its allele frequency was 0.003 (1/388). The remaining two novel variations in the intronic regions (IVS1+71A>G and IVS2−8A>C) were also rare (allele frequency = 0.003 for both).

Three known variations (IVS1+166A>G, IVS3−36C>T and 824T>C) were found at a relatively high frequency (0.106) and were perfectly linked ($r^2 = 1.0$) with each other.

Variations found in the intact *GSTM1* gene and their LD profile: We found 23 variations, including seven novel ones, in 194 Japanese cancer patients (**Table 5**). Ten variations were located in the 5′-flanking region, 2 in the coding exons, 9 in the introns, and 2 in the 3′-flanking region. All detected variations were in Hardy-Weinberg equilibrium ($p > 0.37$ by the χ^2 test or Fisher's exact test) except for 1107+41C>T in the 3′-flanking region ($p = 0.003$ by the Fisher's exact test). Deviation from Hardy-Weinberg equilibrium for this variation was due to 2 more homozygotes than expected among 16 *GSTM1* +/+ subjects.

Seven novel variations, −416G>T and −165A>G in the 5′-flanking region, IVS1+97C>T, IVS1−79G>A, IVS1−78T>A, and IVS2+202G>A in the introns and 1107+128G>A in the 3′-flanking region, were found in single subjects (allele frequencies = 0.003). No novel nonsynonymous SNPs were detected.

Sixteen other variations were already reported or publicized in the dbSNP and/or JSNP databases. They were detected in more than 10 chromosomes (allele frequencies ≥ 0.026) in our population except for −423C>G and IVS2+118T>C (allele frequency = 0.003).

The pairwise |D′| values between 14 common variations (N ≥ 10) in *GSTM1* were higher than 0.95 except for the combinations between −480A>G and other variations, which showed lower |D′| values ($0.27 < |D'| < 1.0$). As for the r^2 values, strong LDs ($r^2 > 0.87$) were observed among 10 variations,

Table 3. Frequencies of *GSTT1* and *GSTM1* deletions

(a)

	Genotype	N	Frequency (%)	Allele	N	Frequency (%)
GSTT1	*0/*0	92	0.474	*0	266	0.686
	*0/+	82	0.423			
	+/+	20	0.103	+	122	0.314
GSTM1	*0/*0	93	0.479	*0	271	0.698
	*0/+	85	0.438			
	+/+	16	0.082	+	117	0.302

(b)

Genotype combination		N	Frequency (%)
GSTT1	*GSTM1*		
*0/*0	*0/*0	45	0.232
	*0/+	42	0.216
	+/+	5	0.026
*0/+	*0/*0	39	0.201
	*0/+	34	0.175
	+/+	9	0.046
+/+	*0/*0	9	0.046
	*0/+	9	0.046
	+/+	2	0.010

*0, deletion; +, intact gene

Table 4. Summary of *GSTT1* SNPs detected in a Japanese population

SNP ID			Location	Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Allele frequency (N = 388)
This study	dbSNP (NCBI)	JSNP		NT_011520.11	From the translational initiation site or from the end of nearest exon			
MPJ6_GTT1001[a]			intron1	3774618	IVS1+71A>G	catagcttagggA/Gacttctcccagc		0.003
MPJ6_GTT1002	rs140313	ssj0002194	intron1	3774523	IVS1+166A>G	gatccaagagtcA/Ggggctccccaaa		0.106
MPJ6_GTT1003[a]			intron2	3770088	IVS2-8A>C	catgacccccacA/Ccccacagtgtgg		0.003
MPJ6_GTT1004[a]			Exon3	3770055	226C>A	ctctacctgacgC/Agcaaatataagg	Arg76Ser	0.003
MPJ6_GTT1005	rs140308		intron3	3767603	IVS3-36C>T	ctaactccctacC/Tccagtaactccc		0.106
MPJ6_GTT1006	rs4630	ssj0002197	3′-UTR	3766891	824(*101[b])T>C	ggaatggcttgcT/Ctaagacttgccc		0.106

[a]Novel variations detected in this study.
[b]The nucleotide that follows the translation termination codon TGA is numbered and starts as *1.

Table 5. Summary of *GSTM1* SNPs detected in a Japanese population

SNP ID			Location	Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Reported alleles	Allele frequency (N=388)
This study	dbSNP (NCBI)	JSNP		NT_019273.18	From the translational initiation site or from the end of nearest exon				
MPJ6_GTM1001	rs412543	ssj0002146	5′-flanking	6137629	−552C>G	agactaagccct**C/G**ggagtagctttc			0.044
MPJ6_GTM1002	rs3815029	ssj0002147	5′-flanking	6137641	−540C>G	gggagtagctttt**C/G**ggatcagaggaa			0.026
MPJ6_GTM1003	rs412302	ssj0002148	5′-flanking	6137701	−480A>G	tcccaggttggg**A/G**ccaccacttttt			0.064
MPJ6_GTM1004	rs3815026		5′-flanking	6137758	−423C>G	cccttgggaact**C/G**ggcagcggagag			0.003
MPJ6_GTM1005[a]			5′-flanking	6137765	−416G>T	gaactcggcagc**G/T**gagagaaggctg			0.003
MPJ6_GTM1006	rs4147561	ssj0002149	5′-flanking	6137783	−398C>T	aaggctgagggac**C/T**accgcgggcagg			0.077
MPJ6_GTM1007	rs4147562	ssj0002150	5′-flanking	6137784	−397A>T	aggctgagggac**A/T**ccgcgggcaggg			0.077
MPJ6_GTM1008	rs4147563	ssj0002151	5′-flanking	6137788	−393T>C	tgagggacaccg**T/C**gggcagggagga			0.080
MPJ6_GTM1009	rs28549287	ssj0002152	5′-flanking	6137823	−358G>A	gagctttgctcc**G/A**ttaggatctggc			0.075
MPJ6_GTM1010[a]			5′-flanking	6138016	−165A>G	cttactgagtgc**A/G**gccccaggcgcc			0.003
MPJ6_GTM1011[a]			intron1	6138313	IVS1+97C>T	tcctcttcaggg**C/T**tgcccgcctcag			0.003
MPJ6_GTM1012[a]			intron1	6138398	IVS1−79G>A	ggtacgtgcagt**G/A**taaactgggggc			0.003
MPJ6_GTM1013[a]			intron1	6138399	IVS1−78T>A	gtacgtgcagtg**T/A**aaactgggggct			0.003
MPJ6_GTM1014	rs4147564	ssj0002153	intron2	6138670	IVS2+118T>C	ctgcaggctgtc**T/C**cttccctgagcc			0.003
MPJ6_GTM1015[a]			intron2	6138754	IVS2+202G>A	ctgtctaattgg**G/A**acgggtgtccct			0.003
MPJ6_GTM1016	rs737497	ssj0002154	intron3	6139277	IVS3−78C>T	cccggtctcctc**C/T**ctgctcttgctt			0.077
MPJ6_GTM1017	rs4147565	ssj0002155	intron4	6139462	IVS4+26A>G	gctgcaatgtgt**A/G**gggggaaggtgg			0.080
MPJ6_GTM1018	rs4147566	ssj0002156	intron5	6139772	IVS5+140C>T	cagttattctca**C/T**gactccaatgtc			0.077
MPJ6_GTM1019	rs1065411	ssj0002159	Exon7	6140823	519C>G	atttgagcccaa**C/G**tgcttggacgcc	Asn173Lys	*B	0.077
MPJ6_GTM1020	rs1056806	ssj0002160	Exon7	6140832	528C>T	caagtgcttgga**C/T**gccttcccaaat	Asp176Asp		0.077
MPJ6_GTM1021	rs4147569	ssj0002161	intron7	6143292	IVS7−221G>A	tgtagaatcttc**G/A**taagtgttagct			0.080
MPJ6_GTM1022	rs4147570	ssj0002162	3′-flanking	6144093	1107(*450)+41C>T[b]	ctggccatctac**C/T**cagactgtctgt			0.026
MPJ6_GTM1023[a]			3′-flanking	6144180	1107(*450)+128G>A[b]	ggattctgctgg**G/A**catagtaaggcg			0.003

[a]Novel variations detected in this study.
[b]The position of the 3′ end of exon 8 (1107 or *450)+the position in the 3′-flanking region. (*450 indicates the position from the termination codon TAG.)

(a) *GSTT1*

Haplotype	Nucleotide change	IVS1+71 A>G	IVS1+166 A>G	IVS2-8 A>C	226C>A Arg76Ser	IVS3-36 C>T	824T>C	Number	Frequency
	Amino acid change								
*0	*0			whole deletion				266	0.686
*1	*1a							78	0.201
	*1b							41	0.106
	*1c							1	0.003
	*1f[§]							1	0.003
*2	*2a[§]							1	0.003
								388	1.000

(b) *GSTM1*

Haplotype	Nucleotide change	-552C>G	-540C>G	-480A>G	-423C>G	-416G>T	-398C>T	-397A>T	-393T>C	-358G>A	-165A>G	IVS1+97 C>T	IVS1-79G>A	IVS1-78T>A	IVS2+118 T>C	IVS2+202 G>A	IVS3-7 4C>T	IVS4+26 A>G	IVS5+140 C>T	519C>G	528C>T	IVS7-221G>A	1107+41 C>T	1107+128 G>A	Number	Frequency
	Amino acid change																			Asn173Lys	Asp176Asp					
*0	*0	whole deletion																							271	0.698
*1	*1a																								54	0.139
	*1b																								10	0.026
	*1c																								10	0.026
	*1d																								6	0.015
	*1e																								2	0.005
	*1f																								1	0.003
	*1g																								1	0.003
	*1h																								1	0.003
	*1i																								1	0.003
	*1j[§]																								1	0.003
*2	*2a																								17	0.044
	*2b																								10	0.026
	*2c																								2	0.005
	*2d[§]																								1	0.003
																									388	1.000

Fig. 1. *GSTT1* (a) and *GSTM1* (b) haplotypes in a Japanese population
Each haplotype is shown in the row, and the alleles are in the columns with the white cell being the major allele and gray cell the minor (nucleotide alteration). [§]Haplotypes were inferred in only one patient and were ambiguous except for the marker SNPs.

−398C>T, −397A>T, −393T>C, −358G>A, IVS3−78C>T, IVS4+26A>G, IVS5+140C>T, 519C>G (Asn173Lys), 528C>T (Asp176Asp), and IVS7−221G>A. Of these variations, two (−398C>T and −397A>T) and four (IVS3−78C>T, IVS5+140C>T, 519C>G, and 528C>T) pairs of SNPs were in perfect LD ($r^2 = 1.0$).

Haplotype estimation and selection of haplotype-tagging SNPs (htSNPs): Based on results of the LD profiles, haplotypes of *GSTT1* and *GSTM1* were analyzed as one LD block that spans at least 7.7 kb and 6.5 kb, respectively. Using the six variations and null alleles in *GSTT1*, three common haplotypes (*GSTT1*0*, **1a* and **1b*) and three rare haplotypes (**1c*, **1d* and **2a*) were identified or inferred (**Fig. 1a**). Frequencies of the common haplotypes, **0*, **1a*, and **1b*, were 0.686, 0.201, and 0.106, respectively. Thus, the htSNPs are either one of IVS1+166A>G, IVS3−36C>T, and 824T>C for **1b* and 226C>A for **2*.

For the *GSTM1* gene, three groups of haplotypes (*GSTM1*0*, **1* and **2*), each containing 1, 10 and 4 subtypes, were identified or inferred using the 23 variations and the null allele (**Fig. 1b**). The **2* group (**2a* to **2d*) was defined as the haplotypes harboring the known nonsynonymous SNP, 519C>G (Asn173Lys), which was previously assigned **B*.[8] The most dominant haplotype was **0* (0.698 frequency), followed by **1a* (0.139), **2a* (0.044), **1b* (0.026), **1c* (0.026), and **2b* (0.026). These six haplotypes accounted for 95% of all haplotypes. The htSNPs that were able to resolve the 5 common haplotypes of the intact genes were −552C>G (**1b* and **1d*), −540C>G (**2b*), −480A>G (**1b* and **2b*), 519C>G (Asn173Lys) (**2*), and 1107+41C>T (**1c*).

Discussion

The present study provides the first comprehensive data on genetic variations of *GSTT1* and *GSTM1* in Japanese, the genes encoding the phase II metabolic enzymes important for cellular defense systems. Moreover, SNPs in intact genes were identified by resequencing, and haplotype structures and tagging SNPs were shown.

It is well recognized that **0* alleles in *GSTT1* and *GSTM1* distribute with different frequencies in several ethnicities. We have shown that 47.4% and 47.9% of our Japanese population homozygously lack *GSTT1* (*GSTT1*0/*0*) and *GSTM1* (*GSTM1*0/*0*), respectively. The *GSTT1*0/*0* frequency is comparable to that reported previously in Japanese (54.0%)[14] and east Asians such as Koreans (46–62%)[7,15] and Chinese (49–58%),[16,17] but was higher than Malay (38%),[17] Indians (16%),[17] Caucasians (15–24%),[7,18] African Americans (22–24%),[7,18] Mexican Americans (10%),[7] and Scandinavians (15%).[7] On the other hand, no marked differences are found in the frequencies of *GSTM1*0/*0* between Caucasians (42–60%)[7,18] and East Asians including Japanese, Koreans

and Chinese (44–63%),[7,14–16] although these frequencies were higher than that of Africans (16–36%).[7,18] The subjects bearing neither *GSTT1* nor *GSTM1* were observed at 23.2%, the frequency of which is similar to Koreans (29.1%)[15] and Shanghai Chinese (24%),[16] but higher than Caucasians (7.5–10.4%)[7,18] and Africans (3.9%).[18]

A number of association studies of the *GSTM1* and *GSTT1* genotypes with cancer susceptibility and cancer therapy outcome have been reported; however, the results are sometimes conflicting.[5–7] In our 194 patients with mainly non-small cell lung cancers, the frequency of *GSTT1*0/*0* and *GSTM1*0/*0* was similar to those in healthy Japanese. This result is in good agreement with a body of literature where the effects of *GSTT1* and *GSTM1* null genotypes on lung cancer development were not clear unless other genetic traits affecting carcinogen metabolism such as *CYP1A1*2A* and *GSTP1*B* (Ile105Val) were combined.[7]

One novel *GSTT1* nonsynonymous variation (226C>A, Arg76Ser) was found in one subject. Arg76 is located in the α3 helix of N-terminal domain I, which forms glutathione binding sites.[19,20] In the crystal structure of human GSTT1-1, this residue closely (2.7 Å) contacts Tyr85 of another subunit (Protein Data Bank, 2C3T).[21] Arg76 is conserved among human, bovine and chicken, whereas this residue is a histidine in mouse and rat. Interestingly, rat and mouse GSTT2 have Ser at position 76.

Of the six SNPs detected in *GSTT1*, three were perfectly linked, resulting in a simple haplotype structure. One of the linked SNPs, 824T>C, was analyzed for various ethnicities in the SNP500Cancer Database (http://snp500cancer.nci.nih.gov/). Its frequency in Japanese (0.106) was comparable to that in Caucasians (0.121), but lower than that in Africans and African-Americans (0.70).

In the *GSTM1* 5′-flanking region (up to −650), eight known SNPs in the NCBI dbSNP database were also detected in this study. This was in contrast to *GSTT1*, in which no SNPs were detected in the 5′-flanking region (up to −801 bp). Murine GSTM1 is transcriptionally upregulated by the Myb proto-oncogene protein through the Myb-binding site (−58 to −63) in the *GSTM1* promoter,[22] whereas no studies on the mechanisms of transcriptional regulation have been performed with human GSTM1. The four common SNPs, −398C>T, −397A>T, −393T>C, and −358G>A (0.075–0.080 in frequencies), were almost perfectly linked with the known SNP, 519C>G (Asn173Lys, *GSTM1*B*) in Japanese. The GSTM1a-1a isozyme (Asn173) and GSTM1b-1b isozyme (Lys173) were reported to have similar catalytic activities *in vitro*.[8] Nevertheless the association of the *GSTM1*A* alleles has been shown with a reduced risk for bladder cancer.[23] Therefore, the functional significance of promoter SNPs on *GSTM1* expression should be further elucidated.

In conclusion, deletions of *GSTT1* and *GSTM1* in Japanese were analyzed by conventional PCR and TaqMan real-time PCR. About one-fourth (0.232 in frequency) of subjects had double *GSTM1* and *GSTT1* null genotypes. In the intact *GSTT1* and *GSTM1* genes, six and 23 SNPs were identified, respectively, and three (*GSTT1*0*, **1a*, **1b*) and six (*GSTM1*0*, **1a*, **2a*, **1b*, **1c* and **2b*) common haplotypes were inferred. Only one rare nonsynonymous SNP (226C>A, Arg76Ser) was found in *GSTT1*, suggesting that this gene is highly conserved. These findings would be useful for pharmacogenetic studies that investigate the relationship between the efficacy of anticancer drugs and *GST* haplotypes.

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Weekly Administration of Epoetin Beta for Chemotherapy-induced Anemia in Cancer Patients: Results of a Multicenter, Phase III, Randomized, Double-blind, Placebo-controlled Study

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Objective: The efficacy and safety of weekly administration of epoetin beta (EPO) for chemotherapy-induced anemia (CIA) patients was evaluated.
Methods: One hundred and twenty-two patients with lung cancer or malignant lymphoma undergoing chemotherapy were randomized to the EPO 36 000 IU group or the placebo group. Hematological response and red blood cell (RBC) transfusion requirement were assessed. Quality of life (QOL) was assessed using the Functional Assessment of Cancer Therapy-Anemia (FACT-An) questionnaire.
Results: Mean change in hemoglobin level with EPO increased significantly over placebo (1.4 ± 1.9 g/dl versus -0.8 ± 1.5 g/dl; $P < 0.001$). The proportion of patients with change in hemoglobin level ≥ 2.0 g/dl was higher for EPO than those for placebo ($P < 0.001$). After 4 weeks of administration, the proportion of RBC transfusion or hemoglobin level < 8.0 g/dl was significantly lower for EPO than those for placebo ($P = 0.046$). The changes in the FACT-An total Fatigue Subscale Score (FSS) were less deteriorated with EPO than those with placebo. Progressive disease (PD) did not influence the change in hemoglobin level but there was less decrease in FSS in non-PD patients. No significant differences in adverse events were observed. Thrombovascular events and pure red cell aplasia related to EPO were not observed. Retrospective analysis of survival showing the hazard ratio of EPO to placebo was 0.94.
Conclusion: Weekly administration of EPO 36 000 IU significantly increased hemoglobin level and ameliorated the decline of QOL in CIA patients over the 8-week administration period.

Key words: anemia – erythropoietin – cancer – chemotherapy-induced anemia – quality of life – survival

INTRODUCTION

One of the causes of anemia in cancer patients is myelosuppression due to chemotherapy or radiation therapy (1). Anemia occurs at a high frequency when using platinum agents, taxanes or anthracyclines often used in cancer patients, especially in patients with lung cancer and malignant lymphomas. Clinical symptoms associated with anemia such as tachycardia, palpitations, fatigue, vertigo and dyspnea are observed in patients with hemoglobin level < 10.0 g/dl, and quality of life (QOL) patients is markedly reduced.

In Japan, only red blood cell (RBC) transfusions have been approved for the treatment of chemotherapy-induced anemia (CIA). However, although the safety of RBC transfusions has improved, there are still concerns about viral infections and graft-versus-host disease, as well as adverse effects on survival prognosis. Erythropoiesis-stimulating agents (ESAs) were approved for the treatment of CIA in the 1990s in the United States and in Europe, but they have still not

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been approved for this indication in Japan. It has been reported that the requirement for RBC transfusion can be reduced and QOL improved by increasing the hemoglobin level by ESA administration (2–7). In the United States, 'Use of epoetin in patients with cancer: evidence-based clinical practice guidelines of the American Society of Clinical Oncology and the American Society of Hematology' (8) (the ASH/ASCO guidelines) was published in 2002. The present placebo-controlled, double-blind, comparative study was planned in 2003 based on the ESAs guidelines and applications for ESAs in the United States and Europe for reference. Since 2003, however, several clinical studies have reported that ESAs worsened prognosis in cancer patients (9–16), and the risks of ESAs were investigated by three meetings of the Oncologic Drugs Advisory Committee (ODAC) (May 2004, May 2007 and March 2008). Since 2007, a safety alert (17) including a change in the upper hemoglobin limit has been issued, and the package inserts have been revised. The ASH/ASCO guidelines were also revised in 2007 (18). The effects of ESAs on cancer patient prognosis are not clear at present, and the US Food and Drug Administration (FDA) revised the labeling for ESAs following the 13 March 2008 ODAC's recommendations to impose additional restrictions.

As a result of a previous dose-finding study, once a week epoetin beta (EPO) 36 000 IU was recommended for Japanese cancer patients (19). In this prospective, placebo-controlled, double-blind comparative study, the efficacy and safety of weekly administration of EPO 36 000 IU was evaluated. Efficacy was assessed based on the hematological response and QOL. In addition, considering the recent regulatory conditions in the United States and in Europe, a survival survey was retrospectively performed, and survival in the EPO group and in the placebo group was compared.

PATIENTS AND METHODS

Patient population

The study protocol was approved by the institutional review board at each study site, and written informed consent was obtained before study-related procedures were begun. Patients eligible for this study were required to be patients of age $\geq$20 to $<$80 years, who had lung cancer or malignant lymphoma, were receiving a platinum-, taxane- or anthracycline-containing chemotherapy regimen with at least two cycles of chemotherapy scheduled after the first study drug administration and had CIA (8.0 g/dl $\leq$ hemoglobin level $\leq$ 11.0 g/dl), an Eastern Cooperative Oncology Group performance status (PS) $\leq$2, life expectancy $\geq$3 months as well as adequate renal and liver function. Exclusion criteria included iron-deficiency anemia (serum iron saturation $<$15% or mean corpuscular volume (MCV) $<$80 μm^3), history of myocardial, pulmonary or cerebral infarction, severe hypertension beyond control by drug therapy, pregnancy, obvious hemorrhagic lesions or other severe complications, myeloid malignancy or ESA/RBC transfusion within 4 weeks before the first study drug administration.

Study design

Patients were randomized 1:1 to receive EPO 36 000 IU or placebo subcutaneously once a week for 8 weeks. The planned number of patients was 120 (60 in each group). Randomization was conducted by central registration system and a dynamic balancing method using tumor type, PS, age and institution as the adjusting factors. Administration was terminated if the hemoglobin level reached 14 g/dl or more. Oral iron-supplementing drugs were administered if serum iron saturation fell below 15% or MCV fell $<$80 μm^3. Hemoglobin level and clinical laboratory tests were monitored weekly until 1 week after last study drug administration. RBC transfusion was allowed at the discretion of the investigator during the study.

Study endpoints

The primary endpoint was change in hemoglobin level from baseline, and the last evaluation was performed 8 weeks after the first study drug administration or at study discontinuation. The last observation carried forward method was used for evaluation of the change in hemoglobin level. The secondary endpoints were change in the Functional Assessment of Cancer Therapy Anemia total Fatigue Subscale Score (FSS) (0–52, where a higher score means less fatigue) from baseline to last evaluation, RBC transfusion requirement, nadir hemoglobin level, proportion of patients who achieved a hemoglobin level increase $\geq$2.0 g/dl from baseline, proportion of the patients with hemoglobin level $<$8.0 g/dl during the study and incidence of either RBC transfusion or hemoglobin level $<$8.0 g/dl. Safety was assessed by National Cancer Institute – Common Toxicity Criteria, ver. 2, translated by the Japan Clinical Oncology Group. Anti-erythropoietin antibodies were measured by enzyme-linked immunosorbent assay and radioimmunoprecipitation assay, and compared with the data of the first study drug administration with the data of the last observation. Detection by either method was judged as positive. A retrospective analysis of survival was performed.

Statistics

Efficacy analyses were performed using the full-analysis-set (FAS) population, comprising all eligible patients who received a study drug. In both EPO and placebo groups, changes in hemoglobin level and changes in FSS at the last evaluation were compared using Student's *t*-test. Stratified analyses in the groups with baseline FSS $>$36 and $\leq$36, respectively, were also performed.