Knowledge of Efficacy of Treatments in Lung Cancer Is Not Enough, Their Clinical Effectiveness Should Also Be Known

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The benefits established in efficacy trials, usually randomized, controlled trials conducted under highly controlled circumstances with maximized internal validity, can frequently not be demonstrated in clinical practice at the community level. Effectiveness trials are tools to evaluate the applicability of a treatment in a wider setting with maximized external validity, to observe uncommon adverse events, and to identify factors influencing the main outcomes and risks. Important areas in relation to lung cancer treatment that will benefit from effectiveness trials include gefitinib monotherapy and bevacizumab therapy combined with cytotoxic chemotherapy for advanced non-small cell lung cancer. These therapies were found to produce life-threatening nonhematologic toxicity at a high incidence of up to 5%; however, the risk factors for these toxicities have not yet been fully established. Effectiveness trials of adjuvant chemotherapy after surgery with long-term follow-up are also important to obtain reliable information as to secondary malignancy and noncancer-related deaths. Development of an infrastructure for effectiveness trials is crucial because of the necessity to deal with large numbers of patients, sometimes as many as 10,000 patients, from many hospitals. The extensive research time involved and the considerable cost of these trials may be reduced with the use of Internet resources. Effectiveness trials are a fundamental step toward bridging the gap between clinical research and clinical practice and effectively implementing new therapies in clinical practice.

Key Words: Efficacy, Effectiveness, Large-scale trials, Lung cancer, Treatment.

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The current paradigm in medical practice is "evidence-based medicine," which has been defined as the "conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients."[1] Randomized, controlled trials (RCTs) are considered the best evidence of efficacy because they employ an experimental design that reduces bias and confounding. The tacit assumption is that the potential benefits of new therapies as shown in RCTs will also be observed in clinical practice. The benefits established in RCTs, however, have been scarcely demonstrated in clinical practice in the community. The response to and compliance with a treatment can be highly dependent on factors such as the patient characteristics, the methods of application of the treatment, and the treatment setting. RCTs are usually performed on a homogeneous study population from which clinically complex patients such as the elderly and infirm patients are generally excluded for the sake of study feasibility. Evidence from such highly selected populations, therefore, cannot easily be generalized to nonselected patients.[2,3]

SUBGROUP ANALYSES AND META-ANALYSES

Subgroup analyses are an approach to enable the most effective use of treatment in routine practice. These analyses may be useful to compare the treatment effects and the risk of adverse events between subgroups in relation to patient characteristics, leading to identification of subgroups of patients most likely to benefit.[4] In this case, the limitations are lack of power due to the smaller number of patients involved, the limits of nonrandomized comparison, and false-positive results from the multiplicity of subgroups, and, therefore, validating the results of such analysis is needed in future trials.[4] Meta-analyses of RCTs aim to integrate the effects of treatment across trials in such a way that they can be translated into practice. Comparing the outcomes of patient subgroups within a meta-analysis may be more useful than a subgroup analysis within a trial, although analyses of individual patient data from trials are necessary.[5] In addition, a meta-analysis has a better external validity than an RCT if the benefit of a treatment was shown on RCTs performed in different settings, in different patient populations, and in different areas of the world.[6]

These methods can evaluate heterogeneity of results from subgroups of patients registered in RCTs, but cannot evaluate patients excluded from these trials, such as patients with comorbidities. Thus, another type of large trial that includes these patients, called effectiveness trials, is needed to apply the treatment in the real world of clinical practice.

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EFFICACY AND EFFECTIVENESS TRIALS

Efficacy and effectiveness are terms that are rarely used correctly and are often interchanged.[7,8] Efficacy is the true biological effect of a treatment under the ideal conditions of an investigation, whereas effectiveness is the beneficial effect observed when the treatment is used in clinical practice in the community at large, which is influenced by many aspects, including the patient characteristics and the social health system. Efficacy trials, also called explanatory trials, are primarily developmental tools used to make inferences related to the treatment modality in question (Table 1).[9] The maximum potential benefits that can be derived from a treatment are estimated under ideal, highly controlled circumstances in clinical research settings, usually in RCTs, to establish a causal link between the treatment and the primary outcome with maximized internal validity. Efficacy trials are conducted in a homogeneous group of patients who are carefully selected based on strict eligibility criteria. The sample size is large enough to have adequate power to detect significant effects. Patients are randomly allocated to either the treatment under investigation or a control standard treatment to equalize the distribution of potential confounding factors. In efficacy trials, the treatment is delivered by highly skilled, rigorously trained, and closely supervised specialists, using standardized, manual-based protocols under close monitoring to ensure fidelity or delivery of treatment as intended in teaching hospitals.

On the other hand, effectiveness trials, which have been called pragmatic, large-scale, or public health trials, are tools to evaluate the applicability of a treatment in a wider setting, to observe uncommon adverse events, and to identify factors influencing the main outcomes and risks (Table 1).[9] To maximize the external validity, or generalizability, effectiveness trials are conducted under naturalistic circumstances in clinical practice settings. Heterogeneous patients selected based on nonstringent eligibility criteria receive the broadly defined treatment without close monitoring or supervision with corrective feedback. The range of the heterogeneity should be as wide as that seen in clinical practice. Inclusion of atypical patients and those with comorbidities will ensure that patients to whom the treatment will be given in the clinical setting will be represented. Large-scale trials may fail to detect a benefit in a population mixed with groups of patients that benefit from the treatment and other groups in which the treatment has no effect or is harmful. It is thus essential to study factors predictive of the treatment effect and to have enough power to perform them. It is very important to identify the population of patients that benefits from the treatment.

The use of stratification is only to improve the power of the analysis and to limit bias in the comparison of subgroups, but not to avoid imbalance in prognostic factors as they are balanced in large trials. The follow-up period is often longer in effectiveness trials.

Although the study design used is often still that of a RCT for these trials, single-arm cohort studies may also be equally, and even sometimes more, appropriate.

HYPOTHESIS AND STUDY DESIGN OF EFFECTIVENESS TRIALS

In contrast to efficacy trials, of which the RCT is widely accepted as the standard procedure, the nature of what constitutes sound effectiveness trials is much less clear, and a few study designs have been tried according to their purpose. The hypothesis to be examined in effectiveness trials is the reproducibility of the results of an efficacy trial conducted under a controlled environment in the clinical practice setting. To confirm a hypothesis verified in an efficacy trial that "Treatment A" is better than "Treatment B," an RCT design may also be required in the subsequent effectiveness trials. Several confounding factors should be stratified at randomization, and the sample size may need to be larger than that in the relevant efficacy trial to detect small significant differ-

TABLE 1. General Characteristics of Efficacy and Effectiveness Trials

Characteristics	Efficacy (Explanatory) Trials	Effectiveness (Pragmatic) Trials
Need	To understand a therapeutic process	To make clinical decisions
Purpose	To demonstrate the efficacy in as short a time aspossible	To assess risk, effectiveness, and cost-effectiveness; to identify influencing factors
Focus of inference	Internal validity	External validity, generalizability
Setting	Highly controlled and specificclinical research setting	Less controlled and representativeclinical practice setting
Design	RCTs	Cohort studies or RCTs
Treatment	Clearly defined, manual based	Broadly defined, easily adaptable to the practice setting
Eligibility criteria	Strict	Relaxed
Study population	Homogeneous	Heterogeneous
No. of patients	<1000	1000–10,000
Monitoring	Close supervision with corrective feedback	Not close
Data	Complex and detailed	Simple
Clinician	Rigorously trained	Variable level of training
Institute	Academic hospital	Community hospital

RCTs, randomized controlled trials. Adapted from Nash JM, McCrory D, Nicholson RA. Efficacy and effectiveness approaches in behavioral treatment trials. *Headache* 2005:45:507–512 and Piantadosi S . *Clinical Trials*. New York: John Wiley & Sons, Inc., 1997.

ences in a heterogeneous patient population. In contrast, to confirm the efficacy of "Treatment A," such as the response and survival obtained in an efficacy trial, a prospective, single-arm cohort design may be adequate for the subsequent effectiveness trial. Diversity in patient population and setting should be enhanced by using practice-oriented protocols to reduce barriers to participation to identify prognostic factors. The primary end point in these trials, for example, the 2-year survival rate, should be evaluated in subset groups of patients categorized by prognostic factors as well as in a whole population. Because of its higher potential for bias than RTCs, detailed description of the cohort constitution and of the patients excluded from it should be included.

A meta-analysis of large RCTs with long-term follow-up can be used to evaluate harmful effects, but are not optimal to detect rare toxicities. To study acute and late toxic effects, several designs are possible: prospective cohorts, health insurance/claim databases, and cancer registries. Prospective cohort studies of combination chemotherapy and combined modality therapy are good candidates for investigator-initiated trials. In the framework of the new drug development, the efficacy and effectiveness are evaluated mainly in phase III and IV trials, respectively. Phase IV trials are conducted after obtaining approval for the drug use to monitor the safety and effectiveness in the general population. Rare, but life-threatening adverse events of a drug (e.g., interstitial lung disease [ILD]) or a combination of drugs (e.g., combination of the antiviral agent sorivudine and oral fluorouracil analogues) may be identified in this phase.[10,11] The Ministry of Health, Labor, and Welfare of Japan recently approved some new drugs on the condition that their toxicity is prospectively surveyed in the clinical setting. These include leflunomide, a newly developed disease-modifying antirheumatic drug that exhibits anti-inflammatory, antiproliferative, and immunosuppressive effects, and oxaliplatin for colorectal cancer. According to a recent report of a prospective postmarketing surveillance, of 5506 patients receiving leflunomide between August of 2003 and July of 2005, 76 patients (1.4%) had suspected ILD and 25 died of it, whereas the incidence of ILD associated with leflunomide reported from outside Japan is only 0.02%.[12] This high frequency of ILD among Japanese patients was revealed only by an effectiveness trial.

EFFECTIVENESS TRIALS RELATED TO LUNG CANCER TREATMENT (TABLE 2)

Gefitinib is an orally available, selective epidermal growth factor receptor tyrosine kinase inhibitor that has been shown to exert antitumor activity in patients with previously treated advanced non-small cell lung cancer (NSCLC). The safety and tolerability of gefitinib have been established in four open-label, multicenter, phase I dose-escalation studies and two multicenter, randomized phase II studies. After this drug was marketed in Japan, however, an unexpectedly high incidence of ILD, as high as 5%, was noted in subjects treated with the drug.[10,13] A prospective survey of gefitinib toxicity in 3354 patients with NSCLC treated at 698 hospitals in Japan between June and December of 2003 showed that the incidence of ILD was 5.8% and the mortality was 2.5%. This study also disclosed risk factors for the development of ILD in the Japanese population, including preexisting pulmonary fibrosis, smoking history, and poor performance status.[14] This is an example of the importance of an effectiveness trial for lung cancer treatment.

Bevacizumab, a humanized monoclonal antibody that inhibits vascular endothelial growth factor, has been shown to improve survival when given together with chemotherapy in patients with advanced nonsquamous NSCLC. However, grade 3/4 bleeding from the primary site, central nervous system, gastrointestinal tract, and other organs was noted in 4.5% of patients receiving the drug in a phase III study.[15] These new types of treatment agents with previously uncommon life-threatening toxicity are also considered important areas for effectiveness trials.

Another subject for effectiveness trials may be chemoradiotherapy for locally advanced lung cancer because the superiority of the concurrent over the sequential approach

TABLE 2. Important Areas Related to Lung Cancer Treatment for Effectiveness Trials

Therapy	Subject Population	Toxicity	Incidence
New agent with life-threatening toxicity			
EGFR inhibitors	Advanced NSCLC	Pneumonitis	1%–5%
VEGF inhibitors	Advanced NSCLC	Bleeding	5%
Intensive therapy with life-threatening toxicity			
Chemoradiotherapy	Stage III NSCLC	Pneumonitis	1%–4%
	Limited SCLC	Septic shock	1%
Intensive chemotherapy	Extensive SCLC	Septic shock	1%–2%
Treatment that requires long-term follow-up			
Adjuvant chemotherapy	Stage IB–IIIA NSCLC	Secondary malignancy	Rare
PCI	Limited SCLC	Neurocognitive disturbance	Rare
Treatment for heterogeneous populations			
Chemotherapy for the elderly	Advanced SCLC, NSCLC	Depends on general condition	

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PCI, prophylactic cranial irradiation; SCLC, small-cell lung cancer; VEGF, vascular endothelial growth factor.

was demonstrated only in patients in good general condition.[16,17] How widely applicable concurrent chemoradiotherapy is in the general patient population remains unknown. In addition, evaluation of late toxicities, including secondary malignancies related to smoking and treatment, and neurocognitive disturbance associated with prophylactic cranial irradiation has become more important as more long-term survivors are expected among these patients.[18,19]

Large-scale RCTs in patients with completely resected stage I–IIIA NSCLC aimed to confirm the effect of cisplatin-based adjuvant chemotherapy suggested by the meta-analysis in 1995.[20,21] Only effectiveness trials with long-term follow-up give reliable information as to secondary malignancy and noncancer related deaths in these patients.

Treatment of elderly patients with lung cancer is also an important field of effectiveness trials because many of these patients have comorbidity and decreased organ function, and, consequently, their general condition varies greatly from one patient to another.[22] There is a debate between those who promote age-unspecified large-scale trials with an analysis of the treatment effect according to age as a covariate and those who promote series of trials limited to an elderly population.[23,24] The outcome of the former trials can be generalized only to a small segment of the elderly population who meet the eligibility criteria of trials designed for younger patients, whereas the outcome of the latter trials depends greatly on the definition of the eligibility criteria. Confirmation of effectiveness will be needed in the both types of trials.

INFRASTRUCTURE OF EFFECTIVENESS TRIALS

Development of the appropriate infrastructure for effectiveness trials, which are conducted using a large number of patients, sometimes as many as 10,000 patients, is an urgent task. A central operations office and data coordinating center can handle many aspects of multi-institutional trials, including the recruitment of study institutions, randomization of patients, data collection, data analysis, and quality control. Clinical trials performed in an area with a cancer registry may cost less if collecting the events through a cancer registry without specific follow-up. The difficulty may be more linked to the construction of a network of general hospitals participating actively in clinical research.

The extensive research time and considerable cost of these processes can be reduced with the use of Internet resources.[25] In addition, a study Web site may facilitate communication among the trial personnel. A study Web site may also be used for the following tasks: providing information to potential participants, study subjects, and investigators; listing contact information; and centralizing data handling for patient registration, randomization, and data collection. A news section of the Web site can provide a progress report concerning the trial status and advertise upcoming meetings. A "Frequently Asked Questions" section can provide investigators with answers to common questions regarding the study protocol, and a download page can be a means of distributing study materials (protocol, case report forms, informed consent forms) to participating study centers.[25]

The electronic signature capture technology and electronic data capture system have been developed by several companies, including Fujitsu and Hitachi in Japan. An Internet clinical trial supporting system is now provided in Japan by commercial information technology service providers and the University Hospital Medical Information Network, a cooperative organization for national medical schools in Japan, sponsored by the Ministry of Education, Culture, Science, Sports, and Technology of Japan.[26]

Quality control and quality assurance of clinical trials become more difficult but more important as the numbers of participating hospitals, contributors, and patients grow. Careful study planning, use of information technology for data management, and efficient auditing are critical for effectiveness trials.[27,28] In addition, high-quality study conduct begins with the proper training of all personnel involved in the study.

TRAINING OF CLINICIANS

Efficacy trials are usually conducted by highly trained and experienced clinicians in academic institutes, including university-affiliated hospitals, cancer center hospitals, and central city general hospitals. When clinicians with varying academic backgrounds and levels of training are expected to implement new treatments in routine clinical practice at city hospitals, effective training of these clinicians is essential to bridge the gap between the research and practice environments.[29] However, passive dissemination of information, including via guidelines and didactic lectures, is generally ineffective in altering practices, irrespective of how important the issue or how valid the new treatment might be. Instead, it would seem necessary to use specific strategies to ensure improvements in common clinical practice, including the use of computerized decision support systems, educational outreach visits, and interactive educational meetings that include discussions of practice.[29] Opportunities for these should be provided to clinicians who participate in effectiveness trials.

CONCLUSION

Despite the considerable effort expended on efficacy trials, relatively little attention has been paid to ensure that the potential benefits of a new therapy are reproduced in routine clinical situations. Effectiveness trials are an important step toward bridging this gap and effectively implementing new therapies established in efficacy trials in clinical practice.

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A Literature Review of Molecular Markers Predictive of Clinical Response to Cytotoxic Chemotherapy in Patients with Lung Cancer

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Background: To find candidate genes for a predictive chemosensitivity test in patients with lung cancer by using a literature review.
Methods: Using MEDLINE searches, "in vitro chemosensitivity associated genes" and articles on association of the gene alteration with clinical chemosensitivity in lung cancer patients were selected. We calculated odds ratios (ORs) and their 95% confidence intervals (95% CIs) of response rates for patients who had tumors with or without gene alteration. Combined ORs and 95% CIs were estimated using the DerSimonian-Laird method.
Results: Of the 80 in vitro chemosensitivity-associated genes identified, 13 genes were evaluated for association with clinical chemosensitivity in 27 studies. The median (range) number of patients in each study was 50 (range, 28-108). The response rates of lung cancer with high and low P-glycoprotein expression were 0% and 73% to 85%, respectively ($p < 0.001$). Glutathione S-transferase pi expression (OR 0.22, 95% CI 0.06-0.79), excision repair cross-complementing 1 alterations (combined OR 0.53, 95% CI 0.28-1.01; $p = 0.055$), and tumor suppressor p53 mutation (combined OR 0.25, 95% CI 0.12-0.52) were associated with clinical chemosensitivity.
Conclusion: In total, 80 in vitro chemosensitivity-associated genes were identified in the literature, and high and low P-glycoprotein, glutathione S-transferase pi expression, excision repair cross-complementing 1 alterations, and tumor suppressor p53 mutation were candidates for future clinical trials of chemosensitivity tests in lung cancer patients.

Key Words: chemotherapy, drug response, molecular markers, prediction, lung cancer

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Lung cancer is the leading cause of death in many countries despite extensive basic research and clinical trials. Approximately 80% of patients with lung cancer have developed distant metastases either by the time of initial diagnosis or during recurrence after surgery for local disease. Systemic chemotherapy against lung cancer, however, has limitations in efficacy such that patients with distant metastases rarely live long.[1]

Tumor response to chemotherapy varies among patients, and objective tumor response rates to standard chemotherapy regimens are approximately 20 to 40% in patients with non—small-cell lung cancer and 60 to 90% in patients with small-cell lung cancer. Thus, it would be extremely useful to know in advance whether patients have tumors that respond to chemotherapy agents and whether the tumors would be resistant to such therapy. For this purpose, cell culture-based chemosensitivity tests have been investigated for more than 20 years, but they are not widely accepted because of technical problems such as the large amount of material required, a low success rate for the primary culture, length of time required, and poor correlation with the clinical response.[2–5]

To overcome these obstacles, DNA-, RNA-, and protein-based chemosensitivity tests have been created, but gene alterations that are predictive of the clinical drug response are not established. Recently, as many as 400 genes whose expression was associated with drug response were identified by cDNA microarray studies, but their functions do not seem to be related to drug sensitivity or resistance.[6–10] In addition, the genes identified by microarray studies were highly unstable and depended on the selection of patients used for gene identification.[11,12] The purpose of this study was to provide an overview of gene alterations in lung cancer that are associated with chemotherapy drug response to identify candidate genes for predictive chemosensitivity tests in patients with lung cancer.

MATERIALS AND METHODS

Because one set of genes associated with chemosensitivity is those directly involved in drug resistance mechanisms, we conducted a MEDLINE search for articles on tumor drug resistance published in the years 2001–2003. This search yielded 112 studies, including several review articles. By searching manually through these articles, we identified 134 genes or gene families that may be involved in drug resistance based on their function. We conducted the second MEDLINE searches for papers of in vitro studies on the 134 genes or gene families by using their names as a keyword.

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From the 134 genes, we selected genes that met the following definition of "in vitro chemosensitivity associated genes": 1) alteration of the gene was identified in a human drug-induced resistant, solid tumor cell line; 2) transfection of the gene induced drug resistance; or 3) down-regulation of the gene or its encode protein increased drug sensitivity. In this latter category, we included studies in which the gene expression or function was suppressed by antisense RNA, hammerhead ribozyme, or an antibody against the gene product. We excluded studies in which drugs were used to inhibit function because the specificity of the drug against the target may not have been complete. We performed a third MEDLINE search for articles on the association between the gene alteration and chemosensitivity of lung cancer cell lines by using the name of the gene as a keyword. Articles in which the association was evaluated in 20 or more cell lines were included in this study. Finally, we searched MEDLINE for studies on the association between the gene alteration and clinical drug response in patients with lung cancer by using the name of the gene as a keyword. Articles in which the association was evaluated in 25 or more patients with advanced lung cancer were included in this study. Studies in which gene expression was evaluated with microarray were excluded because result analysis and interpretation of this technique have not been established, as indicated by the fact that the list of genes identified by microarray studies was highly variable without overlap between these gene sets.[11,12] Clinical studies on concurrent chemoradiotherapy were excluded. We constructed 2 × 2 tables from the response data and calculated odds ratios (ORs), their variances, and 95% confidence intervals (95% CIs) for the patients who had tumors with gene alteration relative to those who had tumors without gene alteration. Combined ORs and 95% CIs were estimated using the DerSimonian-Laird method.[13] When a response rate was 0, association with gene alteration was evaluated using the χ^2 test because 95% CIs for ORs cannot be calculated. The name of each gene was standardized according to Human Gene Nomenclature Database of National Center for Biotechnology Information.

RESULTS

Of the 134 genes or gene families found, a gene alteration in drug-induced resistant cells, an increased or decreased resistance in transfected cells, and an altered sensitivity in gene down-regulated cells were reported for 45, 57, and 32 genes, respectively. In total, 80 genes met the definition of "in vitro chemosensitivity associated gene" (Table 1).

Gene alteration was associated with in vitro chemosensitivity in 15 (50%) of 30 studies on 15 (56%) of 27 gene alterations (Table 2). Clinical drug response was evaluated in 27 studies on 13 gene alterations. The methods used to identify gene alteration included immunohistochemical protein expression analysis (n = 18), polymerase chain reaction (PCR)-based mRNA expression analysis (n = 3), and PCR-based mutation analysis (n = 6). All but one clinical study was retrospective, and the median (range) number of patients in each study was 50 (28-108). Gene alteration was associated with clinical response in 8 of the 27 (30%) studies (Table 2).

TABLE 1. In Vitro Chemosensitivity-Associated Genes

Transporters: ABCA2, ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCG2, MVP, ATP7A, ATP7B, SLC29A1, SLC28A1, SLC19A1
Drug targets: TUBB, TUBB4, TUBA, TYMS, TOP1, TOP2A, TOP2B, DHFR,
Target-associated proteins : MAP4, MAP7, STMN1, KIF5B, HSPA5, PSMD14, FPGS
Intracellular detoxifiers: GSTP1, GPX, GCLC, GGT2, MT, RRM2, AKR1B1
DNA damage recognition and repair proteins: HMGB1, HMGB2, ERCC1, XPA, XPD, MSH2, MLH1, PMS2, APEX1, MGMT, BRCA1, GLO1
Cell cycle regulators: RB1, GML, CDKN1A, CCND1, CDKN2A, CDKN1B
Mitogenic signal regulators: ERBB2, EGFR, KRAS2, HRAS, RAF1
Survival signal regulators: AKT1, AKT2
Integrin: ITGB1
Transcription factors: JUN, FOS, MYC, NFKB1
Apoptosis regulators: TP53, MDM2, TP73, BCL2, BCL2L1, MCL1, BAX, BIRC4, BIRC5, TNFRSF6, CASP3, CASP8, HSPB1

We evaluated the association between transporter P-glycoprotein/multidrug resistance 1 (ABCB1) expression and clinical chemosensitivity in four studies. The response rate of lung cancer with high ABCB1 expression was consistently 0%, whereas that for lung cancer with low ABCB1 expression was 73 to 85% (Table 3). Among drug targets, only topoisomerase II-beta (TOP2B) expression was associated with clinical drug response in patients with small-cell lung cancer (OR 0.29, 95% CI 0.09-0.95). The intracellular detoxifier glutathione s-transferase pi (GSTP1) was associated with both in vitro and clinical drug response (OR 0.22, 95% CI 0.06-0.79) (Table 4). DNA repair gene excision repair cross-complementing 1 (ERCC1) alterations were associated with drug response among patients with non—small-cell lung cancer with marginal statistical significance; the combined OR (95% CI) for ERCC1 alteration was 0.53 (0.28-1.01; p = 0.055) (Table 5). Tumor suppressor p53 (TP53) mutation was the only alteration associated with drug response among patients with non—small-cell lung cancer among genes involved in cell cycle and apoptosis. A combined OR (95% CI) for TP53 among patients with non—small-cell lung cancer was 0.25 (0.12-0.52) (Table 6). B-cell CLL/lymphoma 2 (BCL2) and its family protein expression was not associated with clinical drug response (Table 7).

DISCUSSION

We identified 80 in vitro chemosensitivity-associated genes in our literature search. Of these, 13 were evaluated clinically in 27 studies; ABCB1, TOP2B, GSTP1, and ERCC1 expression and TP53 mutation were associated with changes to drug responses among patients with lung cancer.

Classical drug resistance is believed to be the result of molecular changes inhibiting the drug-target interaction. ABCB1, an ATP-binding cassette protein, acts as an energy-dependent transmembrane efflux pump and decreases the intracellular accumulation of anticancer drugs, including anthracyclines, vinca alkaloids, taxanes, and epipodophyllotox-

TABLE 2. Chemosensitivity-Associated Genes and Association with Chemosensitivity

		Association with chemosensitivity					
		In vitro studies (*n*)			Clinical studies (*n*)		
Category	No of Genes	Total	Yes	%	Total	Yes	%
Transporter	15	9	5	55	4	4	100
Drug target	8	2	1	50	5	1	20
Target-associated protein	7	0	0		0	0	
Intracellular detoxifier	7	3	3	100	1	1	100
DNA repair	10	1	1	100	6	0	0
Damage recognition protein	2	0	0		0	0	
Cell cycle	6	4	2	50	2	0	0
Mitogenic signal	5	3	1	33	1	0	0
Survival signal	2	0	0		0	0	
Transcription factor	4	3	0	0	0	0	
Cell adhesion-mediated drug-resistance protein	1	0	0		0	0	
Apoptosis	13	5	2	40	8	2	25
Total	80	30	15	50	27	8	30

TABLE 3. ABCB1 (P-Glycoprotein) and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Expression	Patients (*n*)	RR (%)	Odds ratio
Yeh et al.[30]	Non-small cell	Paclitaxel	IHC	Low	35	80	0
				High	15	0	$p < 0.001$*
Kawasaki et al.[31]	Small cell	CAV or EP	IHC	Low	26	85	0
				High	4	0	$p < 0.001$*
Hsia et al.[32]	Small cell	EP	IHC	Low	37	73	0
				High	13	0	$p < 0.001$*
Savaraj et al.[33]	Small cell	CAV, CEV, or EP	RT-PCR	Low	24	75	0
				High	7	0	$p < 0.001$*
Combined odds ratio for ABCB expression in patients with SCLC: 0							

IHC, Immunohistochemical analysis; RR, response rate; RT-PCR, reverse transcriptase-polymerase chain reaction.
*Calculated using the χ^2 test because the confidence interval cannot be calculated.

ins. Overexpression of this protein gives tumor cells a multidrug resistance phenotype in vitro, which is thought to be associated with clinical chemoresistance.[14] Our review showed that the response rate of tumors with ABCB1 overexpression was 0 in all studies of lung cancer, whereas that for lung cancer tumors with low ABCB1 expression was 73 to 85% (Table 3).

There is a close relationship between drug sensitivity and quantitative and qualitative alterations of the drug's target, including tamoxifen sensitivity and estrogen receptor expression and trastuzumab response and Her-2/neu overexpression in breast cancer,[15] imatinib resistance and BCR-ABL gene amplification and mutations in Philadelphia chromosome-positive leukemias,[16] and imatinib response and KIT gene mutations in gastrointestinal stromal tumors.[17] In all of these cases, the target molecule is a receptor or a mutated tyrosine kinase located at the entry of growth-stimulating signal transduction pathways. Recently, gefitinib, a tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR), has been developed, and two large phase II trials showed a response rate of 18% and 12% in patients with non—small-cell lung cancer who were previously treated with conventional chemotherapy.[18,19] Responses to the drug have been unpredictable, but mutations of the EGFR gene were identified in patients with gefitinib-responsive lung cancer.[20,21] Furthermore, all mutations in these tumors were restricted to the activation loop of the kinase domain of EGFR, which are in distinct contrast to mutations in extracelluar and regulatory domains of EGFR in glioblastoma, which are unresponsive to gefitinib.[22] Thus, molecular developments of structure and function of the targets hold the promise of targeted cancer therapy. The target molecules of many anticancer cytotoxic agents have not been clearly defined; therefore, the relationship between the target molecule status and sensitivity to the agent has not been established. TOP2B expression was associated with drug response in patients with small-cell lung cancer, with a response rate of 71% for high TOP2B expression tumors versus 90% for low TOP2B expression tumors (OR 0.29, 95% CI 0.09-0.95).[23] This result, however, is in contrast with the idea that a higher

TABLE 4. Drug Targets, Intracellular Detoxifier, and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Expression	Patients (*n*)	RR (%)	Odds ratio (95% CI)
Beta-tubulin class III							
Rosell et al.[34]	Non-small cell	Paclitaxel,	Real-time	Low	13	46	0.39
		Vinorelbine	PCR	High	24	25	(0.09-1.62)
Topoisomerase II-alpha							
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Low	65	85	0.65
				High	23	80	(0.20-2.17)
Dingemans et al.[35]	Non-small cell	Platinum-based	IHC	Low	30	47	0.67
				High	8	38	(0.14-3.40)
Topoisomerase II-beta							
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Low	48	90	0.29
				High	35	71	(0.09-0.95)
Dingemans et al.[35]	Non-small cell	Platinum-based	IHC	Low	18	50	0.86
				High	13	46	(0.21-3.58)
Glutathione s-transferase pi							
Nakanishi et al.[36]	Non-small cell	Cisplatin-based	IHC	Low	17	47	0.22
				High	37	16	(0.06-0.79)

CI, confidence interval; IHC, immunohistochemical analysis; PCR, polymerase chain reaction; RR, response rate; CEV, cyclophosphamide, etoposide, and vincristine; EP, etoposide and cisplatin.

TABLE 5. DNA Repair Genes and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Alteration	Patients (*n*)	RR (%)	Odd ratio (95% CI)
Excision repair cross-complementing 1 expression							
Lord et al. [37]	Non-small cell	Cisplatin,	Real-time	Low	23	52	0.38
		gemcitabine	PCR	High	24	36	(0.11-1.26)
Excision repair cross-complementing 1 (ERCC1) polymorphism at codon 118							
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	C/C	54	54	0.61
			Hybridization	C/T or T/T	53	42	(0.28-1.31)
Combined odds ratio (95% C.I.) for ERCC1 alteration in patients with NSCLC0.53 (0.28-1.01, $p = 0.055$)							
Xeroderma pigmentosum group D polymorphism							
At codon 231							
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	G/G	100	48	1.08
			Hybridization	G/A or A/A	8	50	(0.26-4.57)
At codon 312							
Camps et al.[39]	Non-small cell	Cisplatin,	PCR	G/G	18	17	3.33
		gemcitabine	Sequencing	G/A or A/A	15	40	(0.66-16.7)
At codon 751							
Camps et al.[39]	Non-small cell	Cisplatin,	PCR	A/A	22	23	2.04
		gemcitabine	Sequencing	A/C or C/C	16	38	(0.49-8.45)
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	A/A	96	49	0.74
			Hybridization	A/C	12	42	(0.22-2.51)
Combined odds ratio (95% CI) for XPD polymorphism in patients with NSCLC: 1.38 (0.68-2.78).							

CI, confidence interval; PCR, polymerase chain reaction; RR, response rate; NSCLC, non—small-cell lung cancer; XPD, xeroderma pigmentosum group D.

TABLE 6. Cell Cycle Regulators, Mitogenic Signals, Tumor Protein p53, and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Alteration	Patients (*n*)	RR (%)	Odds ratio (95% CI)
Retinoblastoma 1 expression							
Gregorc et al.[40]	Non-small cell	Cisplatin-based	IHC	Low	61	51	0.45
				High	41	32	(0.20-1.03)
Cyclin-dependent kinase inhibitor 1A, p21 expression							
Dingemans et al.[23]	Small cell	CEV, EP	IHC	Low	63	90	0.57
				High	22	71	(0.17-1.92)
Kirsten rat sarcoma 2 viral oncogene homolog mutation							
Rodenhuis et al. [41, *a*]	Aenocarcinoma	Ifosfamide, carboplatin	PCR-MSH	Normal	46	26	0.65
				Mutated	16	19	(0.16-2.70)
Tumor protein p53 (P53) mutation							
Nakanishi et al.[36]	Non-small cell	Cisplatin-based	IHC	Normal	11	45	0.19
				Mutated	29	15	(0.04-0.94)
Gregorc et al.[40]	Non-small cell	Cisplatin-based	IHC	Normal	56	57	0.26
				Mutated	46	26	(0.11-0.62)
Combined odds ratio (95% CI) for P53 mutation in patients with NSCLC: 0.25 (0.12-0.52)							
Kawasaki et al.[31]	Small cell	CAV or EP	IHC	Normal	10	70	1.3
				Mutated	20	75	(0.24-6.96)
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Normal	47	85	0.81
				Mutated	45	82	(0.27-2.45)
Combined odds ratio (95% C.I.) for P53 mutation in patients with SCLC: 0.93 (0.37-2.35).							

CI, confidence interval; IHC, immunohistochemical analysis; PCR-MSH, polymerase chain reaction-mutation specific hybridization; RR, response rate; CEV, cyclophosphamide, etoposide, and vincristine; EP, etoposide and cisplatin.
[a]Prospective study.

TABLE 7. B-Cell CLL/Lymphoma 2 (BCL2) Family Expression and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Expression	Patients (*n*)	RR (%)	Odds ratio (95% CI)
BCL2							
Krug et al.[42]	Non-small cell	Docetaxel, vinorelbine	IHC	Low	26	46	1.75
				High	5	60	(0.25-12.3)
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Low	20	79	1.36
				High	71	85	(0.38-4.86)
Takayama et al.[43]	Small cell	CAV or EP	IHC	Low	17	76	0.50
				High	21	62	(0.12-2.08)
Combined odds ratio (95% CI) for BCL2 expression in patients with SCLC: 0.87 (0.33-2.32)							
BAX (BCL2-associated X protein)							
Krug et al.[42]	Non-small cell	Docetaxel, vinorelbine	IHC	Low	9	56	0.72
				High	19	47	(0.15-3.54)

CI, confidence interval; IHC, immunohistochemical analysis; RR, response rate; CEV, cyclophosphamide, etoposide, and vincristine; EP, etoposide and cisplatin.

expression of topoisomerase II enzymes correlates with greater chemosensitivity in patients with breast cancer.[24]

In addition to genes involved in classical drug resistance, genes that act downstream of the initial damage induced by a drug-target complex are thought to play an important role in chemosensitivity.[25] ERCC1 is a key enzyme in nucleotide excision repair, one of the key pathways by which cells repair platinum-induced DNA damage. High levels of ERCC1 mRNA have been associated with platinum resistance in the treatment of ovarian and gastric cancer.[26,27] The codon 118 in exon 4 of ERCC1 gene is polymorphic with the nucleotide alteration AAC to AAT. Although this base change results in coding for the same amino acid, it may affect gene expression based on the usage frequency of synonymous codons.[28] The associations between drug response and both ERCC1 gene expression and polymorphism at codon 118 in patients with non—small-cell lung cancer have been reported in the literature. A combined OR (95%

CI) for these ERCC1 alterations was 0.53 (0.28-1.01, p = 0.055), although each study failed to show statistical significant association. Thus, ERCC1 may be a candidate for evaluation of the predictability of drug response in future clinical trials.

TP53, which is mutated or deleted in more than half of lung cancer cells, has a remarkable number of biological activities, including cell-cycle checkpoints, DNA repair, apoptosis, senescence, and maintenance of genomic integrity. Because most anticancer cytotoxic agents induce apoptosis through either DNA damage or microtubule disruption, mutated TP53 may decrease chemosensitivity by inhibiting apoptosis or, in contrast, may increase chemosensitivity by impairing DNA repair after drug-induced DNA damage.[29] This review showed that mutated TP53 was associated with poor drug response in patients with non—small-cell lung cancer (Table 6).

No other genes located downstream (including xeroderma pigmentosum group D, retinoblastoma 1, cyclin-dependent kinase inhibitor 1A, Kirsten rat sarcoma 2 viral oncogene homolog, B-cell CLL/lymphoma 2, and B-cell CLL/lymphoma 2-associated X protein) were associated with clinical drug response (Tables 5-7). The association was evaluated for only 8 of 43 *in vitro* chemosensitivity-associated downstream genes; therefore, key genes may be among the remaining 35 genes. Most clinical studies included a limited number of patients with various background characteristics such as tumor stage and chemotherapy regimen administered, which resulted in low statistical power to identify the association. Finally, because all but one study was retrospective, the quality of tumor samples may vary, and it is therefore unclear whether the gene alteration was detected in all samples. Thus, in future prospective clinical studies, the method of tumor sample collection and preservation, as well as immunohistochemistry and polymerase chain reaction-based methods, should be standardized, and the sample size of patients should be determined with statistical consideration.

The recently developed microarray technique enables investigators analyze mRNA expression of more than 20,000 genes at once, and as many as 100 to 400 genes were selected statistically as chemosensitivity-related genes.[6–8,10] Among them, however, only a limited number of genes were functionally related to chemosensitivity, and only ABCB1 and BAX corresponded with the 80 chemosensitivity-associated genes identified in this literature review, which were picked because of their known function and contribution to in vitro chemosensitivity. Thus, it will be interesting to evaluate the role of expression profile of these genes using microarray analysis.

The association between the expression and alterations of genes and clinical drug responses should be studied further in prospective trials. ABCB1, GSTP1, ERCC1, and TP53, and other genes identified by exploratory microarray analyses should be evaluated in those trials. Simple methods to identify gene alterations, such as immunohistochemistry and polymerase chain reaction-based techniques, will be feasible in future clinical trials because of their simplicity, cost, and time. The median number of patients in retrospective studies analyzed in this review was 50 (range, 28-108). In future prospective trials, sample size consideration for statistical power will also be important.

In conclusion, we identified 80 in vitro chemosensitivity-associated genes in a review of the literature; ABCB1, GSTP1, and ERCC1 expression and TP53 mutation were associated with drug responses among patients with lung cancer.

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Interstitial Shadow on Chest CT is Associated with the Onset of Interstitial Lung Disease Caused by Chemotherapeutic Drugs

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Objective: Pretreatment computerized tomography (CT) films of the chest was studied to clarify the influence of interstitial shadow on developing interstitial lung disease (ILD).
Methods: Eligible patients were those lung cancer patients who started to receive first-line chemotherapy between October 2001 and March 2004. Patients who received thoracic radiotherapy to the primary lesion, mediastinum, spinal or rib metastases were excluded. We reviewed pretreatment conventional CT and plain X-ray films of the chest. Ground-glass opacity, consolidation or reticular shadow without segmental distribution was defined as interstitial shadow, with this event being graded as mild, moderate or severe. If interstitial shadow was detected on CT films of the chest, but not via plain chest X-ray, it was graded as mild. Patients developing ILD were identified from medial records.
Results: A total of 502 patients were eligible. Mild, moderate and severe interstitial shadow was identified in 7, 8 and 5% of patients, respectively. A total of 188 patients (37%) received tyrosine kinase inhibitor (TKI) treatment, namely gefitinib or erlotinib. Twenty-six patients (5.2%) developed ILD either during or after chemotherapy. Multivariate analyses revealed that interstitial shadow on CT films of the chest and treatment history with TKI were associated with the onset of ILD.
Conclusions: It is recommended that patients with interstitial shadow on chest CT are excluded from future clinical trials until this issue is further clarified, as it is anticipated that use of chemotherapeutic agents frequently mediate onset of ILD in this context.

Key words: interstitial lung disease – interstitial shadow – chemotherapy – lung cancer – CT

INTRODUCTION

Interstitial lung disease (ILD) is known to be an adverse event in cancer chemotherapy and radiotherapy. Recently, ILD has attracted considerable attention in Japan since the observation that gefitinib caused ILD (1). Gefitinib is a tyrosine kinase inhibitor (TKI) of epidermal growth factor receptor and is active in patients with recurrent non-small cell lung cancer (NSCLC) after platinum-based chemotherapy (2,3). Gefitinib was first approved for the treatment of advanced NSCLC by the Japanese regulatory agencies on 5 July 2002. From August 2002 to April 2003, ~28 000 patients with NSCLC were given gefitinib in Japan. However, 616 patients suffered from ILD and 246 patients died of ILD, according to a report from AstraZeneca. The West Japan Thoracic Oncology Group conducted a retrospective survey to clarify the risk factors related to ILD (4). Out of 1976 patients with NSCLC who received gefitinib across 84 institutions, 91 patients were suspected of having developed ILD. This group also analyzed the patients' background, together with computerized tomography (CT) films of the chest, before treatment and at the onset of ILD in this subcohort. Five experts in thoracic radiology in these extramural reviews diagnosed ILD in 64 patients. Multivariate analysis indicated that the predictive risk factors for the development of ILD were as follows: male, smoking and existence of idiopathic pulmonary fibrosis. However, this group did not review CT films of the chest in all 1976 patients. How much interstitial shadow on chest CT impacts ILD development remains unknown.

ILD has a high associated risk of death, even if steroid therapy resolves ILD temporarily. Furthermore, ILD affects salvage chemotherapy. In cases where patients are at a high risk of developing ILD, anti-cancer drugs that tend to cause ILD should be avoided. Previous analysis often included only those cases developing ILD, but not all cases undergoing chemotherapy (4,5). The frequency of interstitial shadow in

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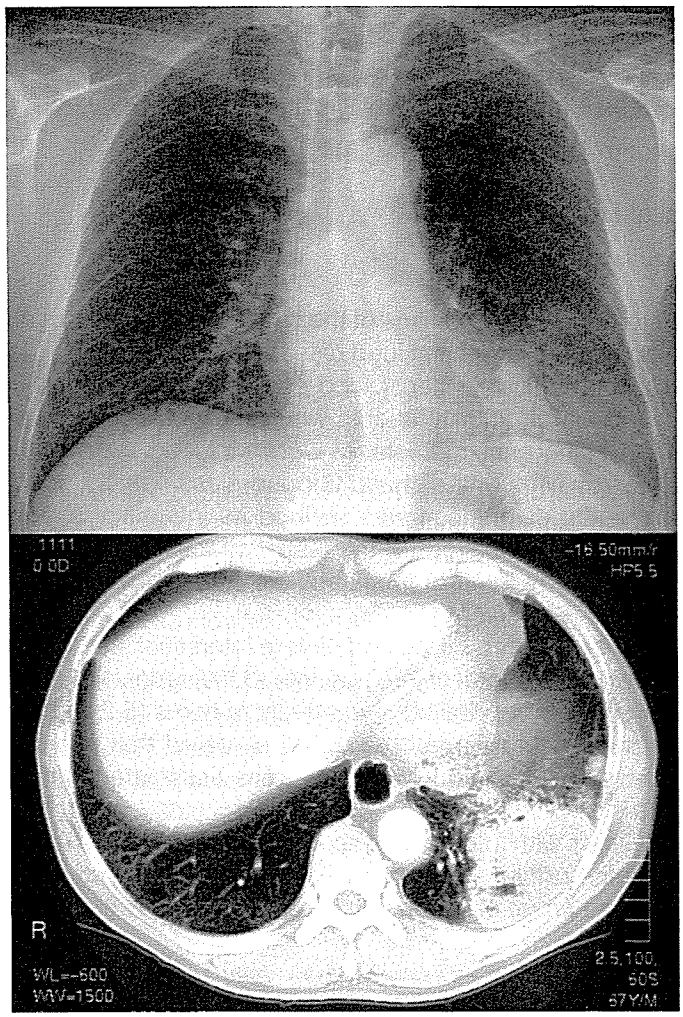

Figure 1. Mild interstitial shadow. An X-ray film of the chest shows no obvious interstitial shadow. A CT film of the chest demonstrated ground-glass opacity in the right basal lung. Interstitial shadow is classified as mild in this case.

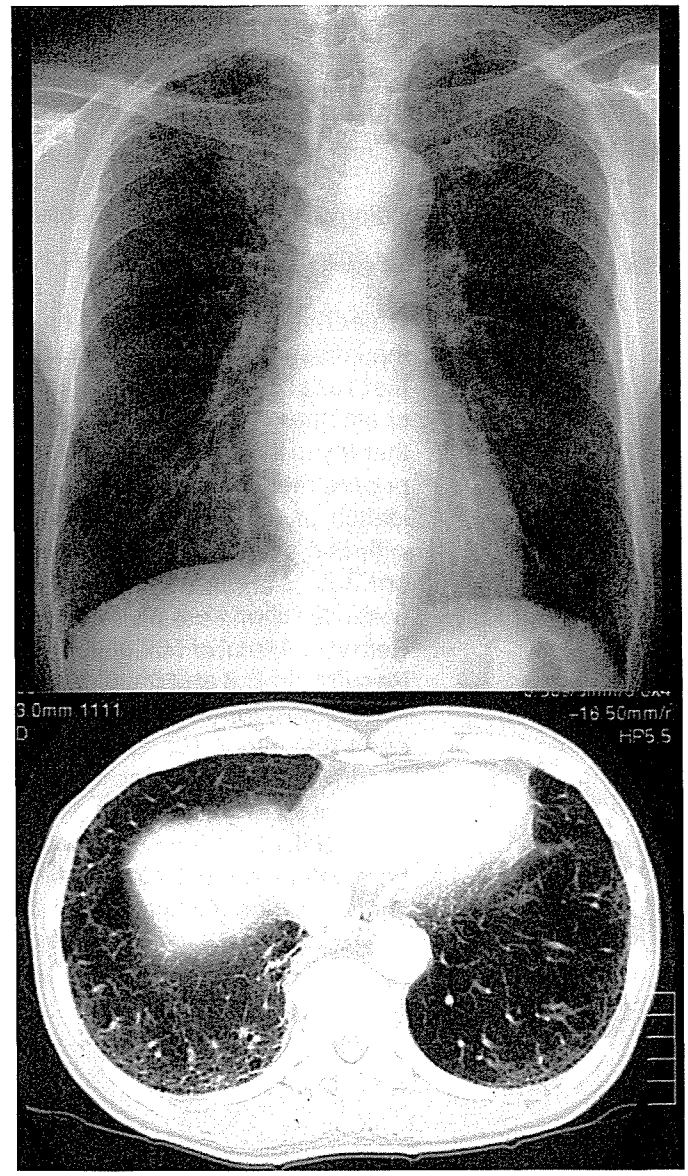

Figure 2. Moderate interstitial shadow. An X-ray film of the chest shows bilateral reticular shadow in the basal area. A CT film of the chest demonstrated bilateral reticular shadow just below the pleura. Interstitial shadow is distributed in 10–30% of the bilateral lower lobes, with this being classified as moderate.

pretreatment CT films of the chest in patients with lung cancer remains unknown, and also how much interstitial shadow confers a risk toward ILD. To further clarify the influence of interstitial shadow on developing ILD, we retrospectively analyzed pretreatment CT films of the chest in consecutive lung cancer patients receiving chemotherapy.

PATIENTS AND METHODS

We retrospectively reviewed the medical records of lung cancer patients who began to receive first-line chemotherapy between October 2001 and March 2004 at the Division of Thoracic Oncology in the National Cancer Center Hospital East. Patients who received thoracic radiotherapy to the primary lesion, mediastinum, spinal or rib metastases were excluded. Plural pulmonologists (S.N., Y.H.K., K.Y., and K.G.) reviewed pretreatment conventional CT and plain X-ray films of the chest. Whether patients had developed ILD or not was blinded to the pulmonologists when they read the films. Conventional spiral CT films were used in our analysis, as high-resolution CT was not routinely conducted. Ground-glass opacity, consolidation or reticular shadow without segmental distribution was defined as interstitial shadow. Localized low attenuation area was defined as emphysema. The grading criteria for interstitial shadow was mild (<10% in bilateral lower lobes), moderate (10–30% in bilateral lower lobes) and severe (>30% in bilateral lower lobes) (Figs 1, 2, and 3). These breakpoints (10 and 30%) were chosen for convenience sake. Interstitial shadow detected on CT films of the chest, but not on plain X-ray, corresponded to mild interstitial shadow. The grading criteria for pulmonary emphysema were mild (<10% in bilateral lungs), moderate (10–30% in bilateral lungs) and severe (>30% in bilateral lungs).

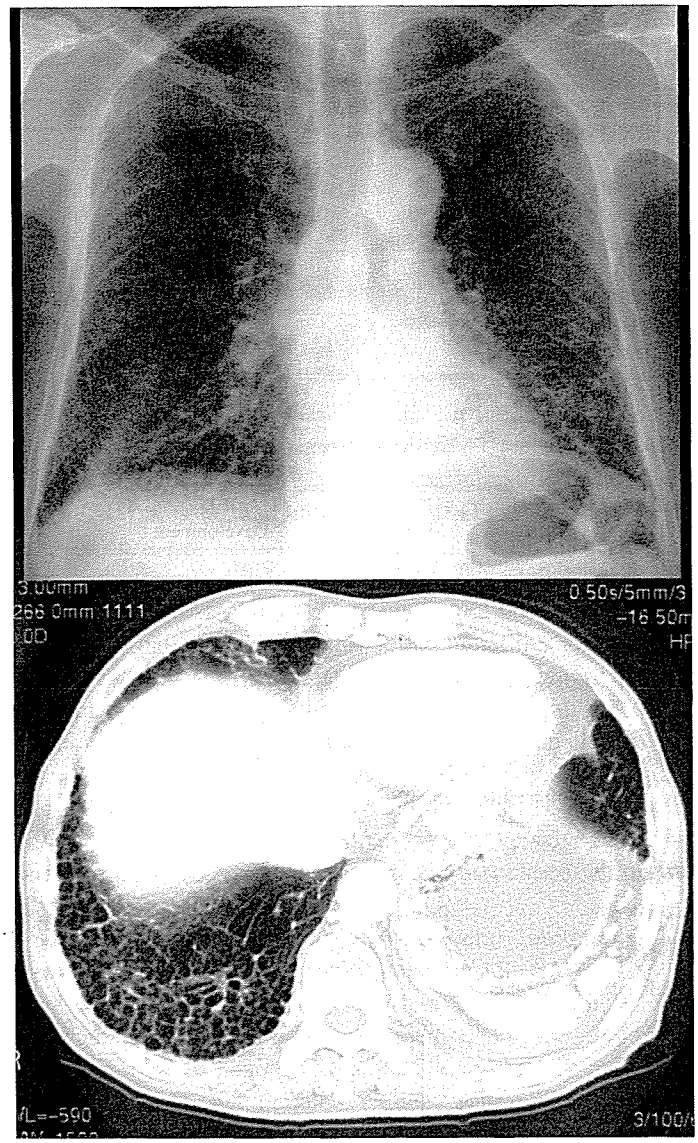

Figure 3. Severe interstitial shadow. An X-ray film of the chest shows bilateral reticular shadow. Reticular shadow is distributed in >30% of the bilateral lower lobes, with this being classified severe.

We identified patients developing ILD, utilizing medical records. ILD was diagnosed on the basis of standard or high-resolution CT findings of the chest (diffuse ground-glass opacity, reticular shadow or consolidation without segmental distribution), elevation of serum levels of lactate dehydrogenase (LDH) and/or KL-6, and lack of response to antibiotics. Bronchoalveolar lavage had not been performed to rule out infections. Most patients diagnosed as ILD were treated with corticosteroids. We compared patients who either had or had not developed ILD in terms of existence and severity of interstitial shadow, emphysema and/or pulmonary bullae on CT films of the chest, as well as patient characteristics including age, gender, smoking history and regimens of received chemotherapy. Comparisons between proportions were performed using a Fisher exact test or a Pearson chi-square test, as appropriate. Multivariate analyses were performed using the logistic regression procedure to determine the relationship between several factors and the onset of ILD.

RESULTS

A total of 502 patients were eligible, with the relevant patient characteristics shown in Table 1. A total of 74% of patients were male and 84% of patients had NSCLC, while the remaining 16% had small cell lung cancer; 79% of the patients were smokers, while 21% never smoked. Platinum-based chemotherapy was performed on 384 patients (76%). A total of 188 patients (37%) received tyrosine kinase inhibitor (TKI) treatment, namely gefitinib or erlotinib. TKI therapy was administered as a first-line (n = 48), second-line (n = 68), third-line (n = 62), fourth-line (n = 9) or fifth-line (n = 1) regimen. Out of 48 patients treated with TKI as a first-line treatment 41 had been entered into a phase II trial of single agent treatment with gefitinib (6).

Radiological findings on this patient cohort are listed in Table 2. Interstitial shadow was detected on chest X-ray and CT in 13 and 20% of patients, respectively. Mild, moderate or severe interstitial shadow was identified in 7, 8 or 5% of patients. Pulmonary emphysema was detected in 38% of patients. Mild, moderate or severe pulmonary emphysema was detected in 18, 10 or 10% of patients. Pulmonary bullae were detected in 20% of patients.

Twenty-six patients (5.2%) developed ILD either during or after chemotherapy. The last regimen of chemotherapy received prior to the onset of ILD included platinum plus vinorelbine or gemcitabine (n = 4), platinum plus taxane (n = 4), other platinum-based chemotherapy (n = 2), vinorelbine plus gemcitabine (n = 2), docetaxel plus gemcitabine (n = 2), single agent treatment with taxane (n = 2) and TKI treatment (n = 10). Out of 26 patients who developed ILD, 14 had a history of taking TKI. Four patients developed ILD after first- or second-line chemotherapy with TKI followed by combination chemotherapy of cisplatin plus vinorelbine (n = 2) or single agent treatment with docetaxel (n = 2).

Univariate analyses demonstrated that male gender (P = 0.0361) and interstitial shadow on CT films of the chest (P = 0.0096) were significantly associated with the onset of ILD (Tables 1 and 3). Multivariate analyses showed interstitial shadow on CT films of the chest [odds ratio (OR): 3.20, 95% confidence interval (CI): 1.34–7.59] and treatment history with gefitinib or erlotinib (OR: 3.17, 95% CI: 1.36–7.36) were associated with the onset of ILD. Male gender was not a significant risk factor for development of ILD in multivariate analysis (OR: 4.33, 95% CI: 0.97–19.38) (Table 4). Univariate and multivariate analyses demonstrated that neither interstitial shadow on X-ray films nor the number of chemotherapy regimens was associated with the onset of ILD.

DISCUSSION

Pulmonary fibrosis or interstitial pneumonia is considered to be a risk factor for ILD caused by drugs (5). In line with the

Table 1. Patient characteristics (n = 502)

	Total	Developed ILD	No ILD Development	P-value
Gender				
Male	371	24	347	0.0361
Female	131	2	129	
Age				
Median (range)	65 (33–83)	66 (53–77)	65 (33–83)	0.5253
ECOG PS				
0–1	443	26	417	0.0590
2–4	59	0	59	
Pathological type				
Adenocarcinoma	279	14	265	0.8775
Squamous cell carcinoma	84	6	78	
Poorly differentiated carcinoma	56	3	53	
Small cell carcinoma	79	3	76	
Others	4	0	4	
Smoking status				
Current smoker	272	14	258	0.1085
Former smoker	124	10	114	
Never smoked	106	2	104	
Clinical stage				
IB	10	0	10	0.6633
IIB	7	0	7	
IIIA	21	0	21	
IIIB	128	8	120	
IV or recurrence after operation	336	18	318	
Treatment history				
Platinum-based	384	18	366	0.3505
Vinorelbine-containing	295	13	282	0.4145
Gemcitabine-containing	110	7	103	0.4758
Taxane-containing	236	14	222	0.5470
Irinotecan-containing	72	2	70	0.5624
Etoposide-containing	67	2	65	0.5573
TKI	188	14	174	0.0954
Number of chemotherapy regimens				
1	212	9	203	0.7733
2	155	9	146	
3	106	7	99	
4 or 5	29	1	28	

ILD, interstitial lung disease; TKI, tyrosine kinase inhibitor.

Table 2. Radiological findings of plain X-ray and computerized tomography films of the chest

Interstitial shadow on plain X-ray films	65 (13%)
Interstitial shadow on CT films	102 (20%)
Mild	37 (7%)
Moderate	42 (8%)
Severe	23 (5%)
Pulmonary emphysema on CT films	189 (38%)
Mild	92 (18%)
Moderate	49 (10%)
Severe	48 (10%)
Pulmonary bullae	101 (20%)

Table 3. Radiological findings and interstitial lung disease

Radiological findings	Developed ILD	No ILD Development	P-value
Interstitial shadow on plain X-ray films of the chest			
No	23	414	1.000
Yes	3	62	
Interstitial shadow on CT film of the chest			
No	15	385	0.0096
Yes	11	91	
Severity of the interstitial shadow			
No	15	385	<0.0001
Mild	8	29	
Moderate	1	41	
Severe	2	21	
Pulmonary emphysema			
No	14	299	0.4075
Yes	12	177	
Severity of the emphysema			
No	14	299	0.6468
Mild	7	85	
Moderate	2	47	
Severe	3	45	
Pulmonary bullae			
No	18	383	0.2052
Yes	8	93	

ILD, interstitial lung disease.

information for prescription, patients with obvious interstitial shadow on chest X-ray should avoid gemcitabine or irinotecan. Although patients with interstitial shadow on chest X-ray were excluded in previous clinical trials in Japan, unexpectedly frequent ILD has been reported, as in the case of combination chemotherapy with docetaxel and gemcitabine (7). Is interstitial shadow on chest X-ray an appropriate criterion to detect interstitial pneumonia or pulmonary fibrosis and avoid ILD? Generally, chest CT can detect interstitial shadow more clearly than chest X-ray. Specifically, high-resolution CT of the chest is essential in diagnosing interstitial pneumonia. However, it has not been determined exactly how much more interstitial shadow detected by CT reveals the onset of ILD. We analyzed CT films of consecutive lung cancer patients who underwent

Table 4. Multivariate analysis of risk factors associated with the onset of interstitial lung disease

Variable	Odds ratio	95% CI	*P*-value
Interstitial shadow on CT films of the chest	3.20	1.34–7.59	0.0086
Treatment history with TKI	3.17	1.36–7.36	0.0073
Male gender	4.33	0.970–19.38	0.0551

CI, confidence interval; TKI, tyrosine kinase inhibitor.

chemotherapy without thoracic radiation therapy. Retrospective review of medical records identified that 26 out of 502 patients developed ILD. We found that interstitial shadow on CT films was associated with onset of ILD, but that interstitial shadow on X-ray was not. We divided interstitial shadow into three classes: mild, moderate and severe. Interstitial shadow on X-ray means moderate to severe interstitial pneumonia. Eight out of 37 patients (22%) with mild interstitial shadow not detected on chest X-ray developed ILD. The reason for the high rate of ILD in patients with mild interstitial shadow is unknown. The criteria of no interstitial shadow on chest X-ray did not sufficiently reduce the risk of ILD. Treatment history with TKI, either gefitinib or erlotinib, was also associated with onset of ILD in multivariate analysis. Conversely, treatment with gemcitabine or irinotecan was not associated with onset of ILD.

Our retrospective analyses have several limitations. We avoided treatment with gemcitabine, irinotecan or TKI in the case of patients with moderate to severe interstitial shadow detectable on chest X-ray films. Some patients who were transferred to another hospital just after chemotherapy may have developed ILD, but detailed clinical courses after transfer were not available. Early death after chemotherapy due to disease progression might conceal the onset of ILD. Although these biases may exist, our analyses were made with an extensive cohort of patients, and therefore the results obtained are of significance.

The frequency of ILD in Japanese patients was reported to range between 3 and 15% in previous clinical trials (6–8). This rate appears to be higher than that observed in the rest of the world. Explanations include the possibility that ILD may be more prevalent among the Japanese or, alternatively, that a greater awareness of the disease could lead to more frequent diagnosis. Furthermore, there may be an increased genetic susceptibility to ILD specifically among the Japanese population (5).

Patients with interstitial shadow on chest X-ray have been excluded in previous clinical trials to avoid ILD caused by chemotherapeutic agents. However, this criterion alone is considered insufficient. It is recommended that patients with interstitial shadow on chest CT are excluded from future clinical trials until this issue is clarified, as it is anticipated that use of chemotherapeutic agents frequently mediate onset of ILD in this context. Therefore, physicians need to understand the associated risk of ILD in patients with interstitial shadow on chest CT and obtain informed consent from patients before administering chemotherapy in clinical practice.

Acknowledgments

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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

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Background: To compare the efficacy and toxicity of three platinum-based combination regimens against cisplatin plus irinotecan (IP) in patients with untreated advanced non-small-cell lung cancer (NSCLC) by a non-inferiority design.
Patients and methods: A total of 602 patients were randomly assigned to one of four regimens: cisplatin 80 mg/m^2 on day 1 plus irinotecan 60 mg/m^2 on days 1, 8, 15 every 4 weeks (IP) carboplatin AUC 6.0 min × mg/mL (area under the concentration–time curve) on day 1 plus paclitaxel 200 mg/m^2 on day 1 every 3 weeks (TC); cisplatin 80 mg/m^2 on day 1 plus gemcitabine 1000 mg/m^2 on days 1, 8 every 3 weeks (GP); and cisplatin 80 mg/m^2 on day 1 plus vinorelbine 25 mg/m^2 on days 1, 8 every 3 weeks (NP).
Results: The response rate, median survival time, and 1-year survival rate were 31.0%, 13.9 months, 59.2%, respectively, in IP; 32.4%, 12.3 months, 51.0% in TC; 30.1%, 14.0 months, 59.6% in GP; and 33.1%, 11.4 months, 48.3% in NP. No statistically significant differences were found in response rate or overall survival, but the non-inferiority of none of the experimental regimens could be confirmed. All the four regimens were well tolerated.
Conclusion: The four regimens have similar efficacy and different toxicity profiles, and they can be used to treat advanced NSCLC patients.
Key words: carboplatin, cisplatin, gemcitabine, irinotecan, non-small-cell lung cancer, paclitaxel, randomized phase III study, vinorelbine

introduction

Nearly 60 000 patients in Japan died of lung cancer in 2004, and the mortality rate is still increasing [1]. Even old-generation cisplatin-based chemotherapy provides a survival benefit and symptom relief in patients with inoperable non-small-cell lung cancer (NSCLC) [2]. Several anticancer agents including irinotecan, paclitaxel, docetaxel, gemcitabine, and vinorelbine, were developed in the 1990s and most of them have mechanisms of action that differ from those of the old-generation agents [3–7]. The combinations of platinum and these new agents developed in the 1990s are more useful against advanced NSCLC than old-generation combination chemotherapy, and doublets of platinum and new-generation anticancer agents are considered standard chemotherapy regimens for advanced NSCLC, although no consistent standard regimens have yet been established [8–17].

Two phase III studies comparing cisplatin plus irinotecan (IP) with cisplatin plus vindesine for advanced NSCLC have been conducted in Japan [18, 19]. Fukuoka et al. [20] reported the results of a combined analysis of the 358 eligible stage IV patients in these studies. They carried out a multivariate analysis using the Cox regression model with adjustment for well-known prognostic factors, and the Cox regression analysis demonstrated that treatment with IP was one of significant independent favorable factor. Based on their data, we selected IP for the reference arm in our study.

The Ministry of Health, Labour and Welfare of Japan approved the prescription of paclitaxel, gemcitabine, and

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vinorelbine for NSCLC in 1999 and requested a phase III study to confirm the efficacy and safety of these agents. The Japanese investigators and the pharmaceutical companies decided to conduct a four-arm randomized phase III study for NSCLC, the so-called FACS, Four-Arm Cooperative Study. The purpose of the study was to compare the efficacy and toxicity of three platinum-based combination regimens, carboplatin plus paclitaxel (TC), cisplatin plus gemcitabine (GP), cisplatin plus vinorelbine (NP), with IP as the reference arm.

patients and methods

patient selection

Patients with histologically and/or cytologically documented NSCLC were eligible for participation in the study. Each patient had to meet the following criteria: clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy, such as malignant pleural effusion, pleural dissemination, malignant pericardiac effusion, or metastatic lesion in the same lobe), at least one target lesion >2 cm, no prior chemotherapy, no prior surgery and/or radiotherapy for the primary site, age 20–74 years, Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, adequate hematological, hepatic and renal functions, partial pressure of arterial oxygen (paO_2) ≥60 torr, expected survival >3 months, able to undergo first course treatment in an inpatient setting, and written informed consent. The study was approved by the Institutional Review Board at each hospital. Written informed consent was obtained from every patient.

treatment schedule

All patients were randomly assigned to one of the four treatment groups by the central registration office by means of the minimization method. Stage, PS, gender, lactate dehydrogenase (LDH) and albumin values, and institution were used as adjustment variables. The first group received the reference treatment, 80 mg/m^2 of cisplatin on day 1 and 60 mg/m^2 of irinotecan on days 1, 8, and 15, and the cycle was repeated every 4 weeks. The second group received 200 mg/m^2 of paclitaxel (Bristol-Myers K.K., Tokyo, Japan) over a 3-h period followed by carboplatin at a dose calculated to produce an area under the concentration–time curve of 6.0 min × mg/mL on day 1 and the cycle was repeated every 3 weeks. The third group received 80 mg/m^2 of cisplatin on day 1 and 1000 mg/m^2 of gemcitabine (Eli Lilly Japan K.K., Kobe, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. The fourth group received 80 mg/m^2 of cisplatin on day 1 and 25 mg/m^2 of vinorelbine (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. Each treatment was repeated for three or more cycles unless the patient met the criteria for progressive disease or experienced unacceptable toxicity.

response and toxicity evaluation

Response was evaluated according to the Response Evaluation Criteria in Solid Tumors, and tumor markers were excluded from the criteria [21]. Objective tumor response in all responding patients was evaluated by an external review committee with no information on the treatment group. Toxicity grading criteria in National Cancer Institute Common Toxicity Criteria Ver 2.0 were used to evaluate toxicity.

quality of life assessment

Quality of life (QoL) was evaluated by means of the Functional Assessment of Cancer Therapy—Lung (FACT-L) Japanese version and the QoL Questionnaire for Cancer Patients Treated with Anticancer Drugs (QoL-ACD), before treatment, immediately before the second cycles of chemotherapy, and 3 and 6 months after the start of treatment [22–24].

statistical analysis and monitoring

The primary end point of this study was overall survival (OS), and the secondary end points were response rate, response duration, time to progressive disease (TTP), time to treatment failure (TTTF), adverse event, and QoL. The 1-year survival rate of the control group in this study was estimated to be 43% based on the data in published papers, and the 1-year survival rate in the other treatment group was expected to be 50%. The lower equivalence limit for 1-year survival rate was set as '−10%'. The criterion for the non-inferiority of each treatment was a lower limit of the two-sided 95% confidence interval (CI) of the 1-year survival rate of treatment minus that of control larger than the lower equivalence limit. Because the non-inferiority of each treatment versus the control was to be evaluated independently, a separate null hypothesis was stated for each treatment, and for that reason no multiple comparison adjustment was included in the study. Based on the above conditions and binomial distribution, 135 patients were needed per arm for a one-sided Type I error of 2.5% and 80.0% power. In view of the possibility of variance inflation due to censoring, the sample size was set at 600 (150 per arm).

Central registration with randomization, monitoring, data collection, and the statistical analyses were independently carried out by a contract research organization (EPS Co., Ltd, Tokyo, Japan).

results

patient characteristics

From October 2000 to June 2002, a total of 602 patients were registered by 44 hospitals in Japan. All patients had been followed up for >2 years, and 447 patients had died as of June 2004. Of the 602 patients registered, 151 were allocated to the reference treatment, IP, and 150, 151, and 150 patients were allocated to TC, GP, and NP, respectively. Since 10 patients did not receive chemotherapy and 11 patients were subsequently found to be ineligible, 592 patients were assessable for toxicity and 581 patients were assessable for efficacy. Four patients did not receive chemotherapy due to electrolytic disorder, fever, symptomatic brain metastases, and rapid tumor progression in IP, two patients due to refusal and pneumonia in TC, four patients due to lower WBC counts (two patients), rapid tumor progression, and nephritic syndrome in NP. Two patents were ineligible due to wrong stage in IP, two patients were wrong stage and one patient had double cancer in TC, two patients were wrong diagnosis, one patient had massive pleural effusion, one patient received prior chemotherapy in GP, one patient had no target lesions in NP. Age, gender, PS, stage, and LDH and albumin values were well balanced in each arm (Table 1). Fewer patients with adenocarcinoma and more patients with squamous cell carcinona were, however, entered in three experimental arms than in IP.

objective tumor response and response duration

Objective tumor response is shown in Table 2. Forty-five partial responses occurred in the 145 assessable patients in the reference arm, IP, for an objective response rate of 31.0% with a median response duration of 4.8 months. The response rate and median response duration were 32.4% and 4.0 months in TC, 30.1% and 3.5 months in GP, and 33.1% and 3.4 months in NP. The response rates in TC, GP, and NP were not statistically different from the rate in IP according to the results of the χ^2 test.

Table 1. Patient characteristics and treatment delivery

	Cisplatin + irinotecan	Carboplatin + paclitaxel	Cisplatin + gemcitabine	Cisplatin + vinorelbine
Assessable patients	145	145	146	145
Gender (male/female)	97/48	99/46	101/45	101/44
Age, median (range)	62 (30–74)	63 (33–74)	61 (34–74)	61 (28–74)
PS (0/1)	44/101	44/101	45/101	45/100
Histology				
Adenocarcinoma	121	104	108	109
Squamous cell carcinoma	16	31	29	29
Others	8	10	9	7
Stage (IIIB/IV)	31/114	28/117	30/116	26/119
No. of cycles				
Mean ± SD	3.0 ± 1.3	3.5 ± 1.5	3.2 ± 1.2	3.1 ± 1.3
Median	3	3	3	3
Range	1–7	1–10	1–7	1–8

PS, performance status; SD, standard deviation.

Table 2. Survival, TTP, TTTF, response rate, and response duration

	N	Median survival months	1-year survival (%)	Difference in 1-year survival from IP	2-year survival (%)	TTP (median) months	TTTF (median) months	Response rate (%)	Response duration (median) months
Cisplatin + irinotecan	145	13.9	59.2	–	26.5	4.7	3.3	31.0	4.8 (n = 45)
Carboplatin + paclitaxel	145	12.3	51.0	−8.2% (95% CI −19.6% to 3.3%)	25.5	4.5 (P = 0.355)[a]	3.2 (P = 0.282)[a]	32.4 (P = 0.801)[b]	4.0 (n = 47)
Cisplatin + gemcitabine	146	14.0	59.6	0.4% (95% CI −10.9% to11.7%)	31.5	4.0 (P = 0.170)[a]	3.2 (P = 0.567)[a]	30.1 (P = 0.868)[b]	3.5 (n = 44)
Cisplatin + vinorelbine	145	11.4	48.3	−10.9% (95% CI −22.3% to 0.5%)	21.4	4.1 (P = 0.133)[a]	3.0 (P = 0.091)[a]	33.1 (P = 0.706)[b]	3.4 (n = 48)

[a]Compared with IP by the generalized Wilcoxon test.

[b]Compared with IP by the χ^2 test.

CI, confidence interval; IP, cisplatin plus irinotecan; TTP, time to progressive disease; TTTF, time to treatment failure.

OS, TTP disease, and TTTF

OS and TTP are shown in Figure 1. Median survival time (MST), the 1-year, and 2-year survival rate in IP were 13.9 months, 59.2%, and 26.5%, respectively. The MSTs, 1-year, and 2-year survival rates were, respectively, 12.3 months, 51.0%, and 25.5% in TC; 14.0 months, 59.6%, and 31.5% in GP; and 11.4 months, 48.3%, and 21.4% in NP. The lower limits of the 95% CI of the difference in 1-year survival rate between IP and TC (−19.6%), GP (−10.9%), and NP (−22.3%) were below −10%, which was considered the lower equivalence limit (Table 2). Thus, the results did not show non-inferiority in three experimental regimens compared with reference treatment. Median TTP and median TTTF were 4.7 and 3.3 months, respectively in IP. Median TTP and TTTF were, respectively, 4.5 and 3.2 months in TC, 4.0 and 3.2 months in GP, and 4.1 and 3.0 months in NP. There were no statistical differences in either TTP or TTTF in TC, GP, or NP, compared with IP according to the results of the generalized Wilcoxon test (Table 2).

hematologic and non-hematologic toxicity

In IP, 47.6% and 83.7% of patients developed grade 3 or worse leukopenia and neutropenia, respectively (Table 3). The incidences of grade 3 or worse leukopenia (33.1%, P = 0.010) and neutropenia (62.9%, P < 0.001) were significantly lower in GP than in IP. The incidence of grade 3 or worse leukopenia (67.1%, P < 0.001) was significantly higher in NP than in IP. Grade 3 or worse thrombocytopenia developed in 5.4% of the patients in IP, and the incidence was significantly higher in GP (35.1%, P < 0.001). The incidence of febril neutropenia in IP was 14.3%, and was significantly lower in GP (2.0%, P < 0.001).

Grade 2 or worse nausea, vomiting, anorexia, and fatigue occurred in 60.5%, 51.0%, 65.3%, and 38.8%, respectively, of the patients in IP. The incidences of grade 2 or worse nausea (TC: 25.0%, P < 0.001, NP: 47.3%, P = 0.022), vomiting (TC: 22.3%, P < 0.001, NP: 36.3%, P = 0.011), and anorexia (TC: 32.4%, P < 0.001, NP: 49.3%, P = 0.005) were significantly lower in TC and NP than in IP. Grade 2 or worse diarrhea was