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original contribution

Cooperative Group Research Efforts in Lung Cancer 2008: Focus on Advanced-Stage Non–Small-Cell Lung Cancer

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Abstract

Clinical trials performed within the cooperative group system play a substantial role in the advancing of lung cancer therapy. Interactions between the leaders of the cooperative groups are critical and occur regularly throughout the year, but the annual Lung Cancer Congress provides a unique forum for representatives from each group to present ongoing and planned studies in an interactive forum. Herein, we highlight discussion from the 9th annual Lung Cancer Congress in June 2008, focused on advanced-stage non–small-cell lung cancer (NSCLC). Many studies are looking at the addition of targeted agents such as bevacizumab, cetuximab, vascular endothelial growth factor receptor inhibitors, and apoptosis-inducing agents to chemotherapy. Personalizing therapy by better selection of patients for particular drugs is also being emphasized, most notably epidermal growth factor receptor fluorescence in situ hybridization overexpression and other predictions of response with cetuximab. Future articles in this series will address early and locally advanced NSCLC as well as other thoracic malignancies such as small-cell lung cancer and mesothelioma. Ongoing trials within the cooperative groups are an essential component of the persistent improvement in the treatment of lung cancer.

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Introduction

The leading cause of cancer-related death worldwide is lung cancer. Though not curative for advanced-stage disease, chemotherapy improves survival and quality of life compared with best supportive care.[1] Combinations of 2 chemotherapy drugs (doublets) are superior to single-drug regimens in response and survival, but adding a third cytotoxic drug increases toxicity with no additional survival benefit.[2] Most doublet regimens include a platinum agent, although a metaanalysis comparing platinum with nonplatinum doublets demonstrated comparable survival but variable toxicity profiles with the various regimens.[3,4] Bevacizumab, an antibody to vascular endothelial growth factor (VEGF), is now approved to be added to the carboplatin/paclitaxel doublet for patients with nonsquamous histology, based on the encouraging results of E4599, a randomized trial led by the Eastern Cooperative Oncology Group (ECOG) that demonstrated a survival benefit when bevacizumab was added to this regimen. This trial highlights the critical role that the North American cooperative oncology groups, sponsored by the National Cancer Institute, and cooperative groups abroad, have played in establishing the current standards of care for non–small-cell lung cancer (NSCLC).[5]

There are 4 general oncology cooperative groups active in lung cancer research within the United States: the ECOG, the Southwest Oncology Group (SWOG), the Cancer and Leukemia Group B (CALGB), and the North Central Cancer Treatment Group (NCCTG). All 4 have member institutions located throughout the country. The National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) oversees cooperative oncology efforts within Canada. As their names imply, the American College of Surgeons Oncology Group (ACOSOG) and the Radiation Therapy Oncol-

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ogy Group (RTOG) are more focused on treatment modality and have member institutions on both sides of the US/Canadian border. The North American cooperative group thoracic leadership, as well as the thoracic heads of international groups such as the European Organization for Research and Treatment of Cancer (EORTC) and the Japanese Cooperative Oncology Group (JCOG), is brought together each year at the annual International Lung Cancer Congress, now in its ninth year. This article will focus on work in advanced-stage NSCLC being performed by each of the cooperative groups represented at the meeting. This series of articles will continue with coverage on the group efforts in earlier stages of NSCLC and in other thoracic malignancies.

American College of Surgeons Oncology Group

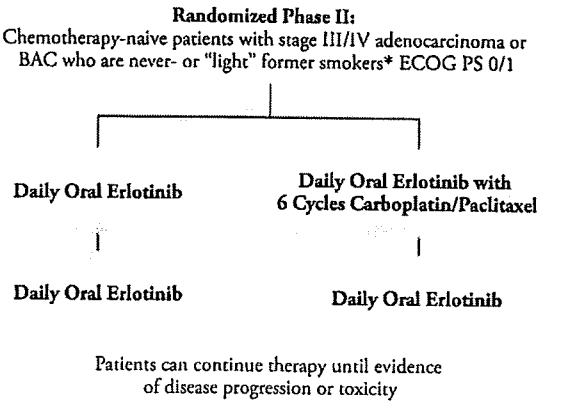
The ACOSOG consists primarily of surgeons throughout North America. The aims of the thoracic committee of this group are to improve local control in early-stage NSCLC and to enhance therapeutic efficacy through biologic and molecular markers. Their contributions to the treatment of patients with advanced-stage disease are therefore limited, but some of the work on markers developed in early-stage disease will likely be translated into work on markers in more advanced-stage disease in the future and are worth noting. The recently completed Z0040 study looked at micrometastases in pleura, bone marrow, and lymph nodes, and outcome correlates in patients with resected early-stage disease. These data have not yet been presented. Z4031 accrued > 1000 patients at 52 sites between 2004 and 2006. Patients with suspicious lung masses were eligible for the trial, which consisted of a preoperative blood draw, resection, then a postoperative blood draw with long-term follow-up. Ongoing analysis is looking at proteomic profiling of the serum as an adjunct to computed tomography scans.

Cancer and Leukemia Group B

Cancer and Leukemia Group B has led several important exploratory trials in advanced-stage NSCLC. There remains considerable controversy about the appropriate care of patients with a poor performance status (PS). CALGB 9730 compared a single agent versus a doublet as first-line therapy and demonstrated that elderly patients derived the same benefit as younger patients, but those with a poor PS had even more benefit with the doublet regimen compared with a single drug.[6] Following up on this, CALGB 30402, led by Rogerio Lilenbaum, MD, enrolled patients with a PS of 2 to receive first-line weekly docetaxel plus cetuximab (an antibody to the epidermal growth factor receptor [EGFR]) or bortezomib (a proteosome inhibitor).[7] After 4 cycles of the doublet, the targeted agent was continued until progression. A total of 30 patients were enrolled on each arm, with a median survival time of 4.4 months versus 3.9 months in the cetuximab-versus-bortezomib arms.

In another approach at incorporating novel agents, but in a broader patient group, the recently completed CALGB 30203 used carboplatin/gemcitabine as a backbone regimen and added zileuton, celecoxib, or both to focus on modulation of the eicosanoid pathway.[8] This exploratory phase II study found that in all groups the overall survival (OS) was very similar, and the study did not meet its goal of a > 50% failure-free survival of 9 months. Immunohistochemistry (IHC) analysis for COX-2, however, found a trend

Figure 1 CALGB 30406 Schema: Erlotinib with or Without Chemotherapy in Nonsmokers



*Never-smoker: ≤ 100 cigarettes/lifetime; "light" former smoker: quit 1 year ago and ≤ 10 pack-years.
Abbreviations: BAC = bronchioloalveolar carcinoma; CALGB = Cancer and Leukemia Group B; ECOG = Eastern Cooperative Oncology Group; PS = performance status

indicating that high levels were a negative prognostic factor for OS but a positive predictor for improved survival with celecoxib. This result has led to discussion of a phase III trial of chemotherapy with or without a COX-2 inhibitor in patients with overexpression of COX-2.

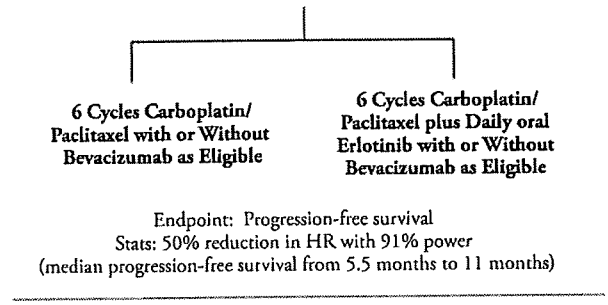
Another planned trial, C30607, will focus on maintenance therapy with the VEGF receptor (VEGFR) tyrosine kinase inhibitor (TKI) sunitinib. Enrolled patients will receive 4 cycles of first-line chemotherapy followed by placebo or maintenance sunitinib.

It has been consistently shown that the EGFR TKIs such as erlotinib provide the best response rates and survival in patients with minimal smoking history.[9] The TALENT and TRIBUTE trials of erlotinib plus first-line chemotherapy failed to show a survival advantage with this approach, but the small number of never-smokers in TRIBUTE did show an OS advantage with the addition of erlotinib (10.1 months vs. 22.5 months with erlotinib).[10] To address this issue further, CALGB 30406 is a randomized phase II study of patients who are newly diagnosed with advanced-stage NSCLC and have smoked < 100 cigarettes in their lifetime (never-smokers) or smoked < 10 pack years in their lifetime and quit over 1 year ago (light smokers; Figure 1). Patients will be randomized to receive daily oral erlotinib until progression of disease or 6 cycles of carboplatin/paclitaxel plus erlotinib followed by erlotinib. This trial will also include extensive correlatives evaluating EGFR expression by IHC, EGFR mutation status, EGFR gene copy number by florescent in situ hybridization (FISH), K-*ras* mutational status, and proteomic analysis.

Eastern Cooperative Oncology Group

The ECOG has played a critical role in defining the standard of care for patients with advanced-stage NSCLC. E1594 randomized patients to 1 of 4 different platinum-based doublets and found them all to be equivalent, thus solidifying the notion that improvements in the treatment of this disease are unlikely to come from further trials of various chemotherapy combinations and

Figure 2 ECOG 2507 Schema: Chemotherapy with or Without Erlotinib in Nonsmokers



*Never-smoker: ≤ 100 cigarettes/lifetime; "light" former smoker: quit ≥ 1 year ago and ≤ 10 pack-years.
Abbreviations: BAC = bronchioloalveolar carcinoma; ECOG = Eastern Cooperative Oncology Group; HR = hazard ratio; PS = performance status

establishing carboplatin and paclitaxel as the "standard" doublet in the United States because of the favorable toxicity profile.[11] E4599, which studied carboplatin/paclitaxel with or without the anti-VEGF antibody bevacizumab, was the first trial to show a survival advantage with the addition of a "targeted" agent to first-line doublet chemotherapy.[12] Median survival time improved from 10.3 months to 12.3 months with the addition of bevacizumab, but the trial was restricted to patients without squamous cell histology, brain metastasis, anticoagulant use, or history of gross hemoptysis. E4599 led to the approval of bevacizumab in combination with carboplatin and paclitaxel as first-line therapy for advanced-stage NSCLC in patients meeting the eligibility criteria. The ECOG has set this combination as its reference regimen and has been looking for ways to build on this platform.

The current protocol in development within ECOG randomizes patients who have completed 4 cycles of carboplatin/paclitaxel/bevacizumab to bevacizumab alone, pemetrexed alone, or a combination of the 2. This trial is seeking to better define the role of maintenance bevacizumab and chemotherapy. The maintenance chemotherapy question has come to the forefront with 2 recent trials showing a trend toward a survival benefit with this approach.[13,14]

Another trial that will build on the E4599 platform is focused on never-smokers. Previously untreated patients with newly diagnosed NSCLC who are never-smokers will be eligible for E2507 (Figure 2). They will be randomized to carboplatin/paclitaxel/bevacizumab with or without erlotinib. Patients who are ineligible for bevacizumab will be randomized to chemotherapy alone with or without erlotinib. This trial is asking a similar question to CALGB 30406, but in the ECOG 2507 trial, all patients receive chemotherapy with randomization to erlotinib or no erlotinib, whereas in the CALGB 30406, all patients receive erlotinib with randomization to chemotherapy or no chemotherapy.

Further work with erlotinib in first-line therapy of NSCLC will be performed in E3503, a protocol in development that builds on work with a proteomic analysis that predicts for response to erlotinib.[15]

The ECOG presented an important positive trial of the VEGFR TKI sorafenib as ≥ third-line therapy for patients with advanced-stage NSCLC at the 2008 meeting of the American Society of Clinical Oncology (ASCO). This trial, E2501, enrolled patients in a "randomized discontinuation" regimen that enriched for patients with stable disease (SD).[16] All enrolled patients received 8 weeks of sorafenib, and those with SD were then randomized to continue on drug versus placebo. Patients with rapidly progressive disease before 8 weeks were discontinued before randomization, and patients with a documented response were continued on therapy without randomization. For patients randomized, the median survival time showed a trend in favor of sorafenib at 11.9 months compared with 9 months for patients on placebo, ($P = .18$; hazard ratio [HR], 0.67; 95% CI, 0.37-1.21). The ECOG is considering a follow-up trial to build on those encouraging results.

European Organization for Research and Treatment of Cancer

Multiple cooperative groups exist in Europe, mostly based by country, but the EORTC spans multiple countries and has been a major contributor to critical trials in lung cancer. In addition to a discussion of ongoing lung EORTC trials, at the Lung Cancer Congress, Paul Baas, MD, PhD, highlighted other ongoing European trials in advanced-stage lung cancer. These other trials ongoing in Europe include a first-line trial with erlotinib and bevacizumab, a randomized phase II of mistletoe as a complementary treatment in advanced-stage NSCLC, and a phase III trial of vandetanib in patients who have not responded to therapy with other EGFR TKIs.

The EORTC trials include EORTC 08021, a randomized phase II study in conjunction with the Italian Lung Cancer Project that randomizes patients to immediate gefitinib versus placebo after completion of standard first-line chemotherapy for advanced-stage NSCLC. Eligible patients have advanced-stage NSCLC, with a PS of 0-2 and strong EGFR expression. They must receive 2-6 cycles of a platinum-containing doublet, and if they are without progression, they are randomized to gefitinib or placebo. The planned sample size is 450, of which about half had been enrolled as of June 2008.

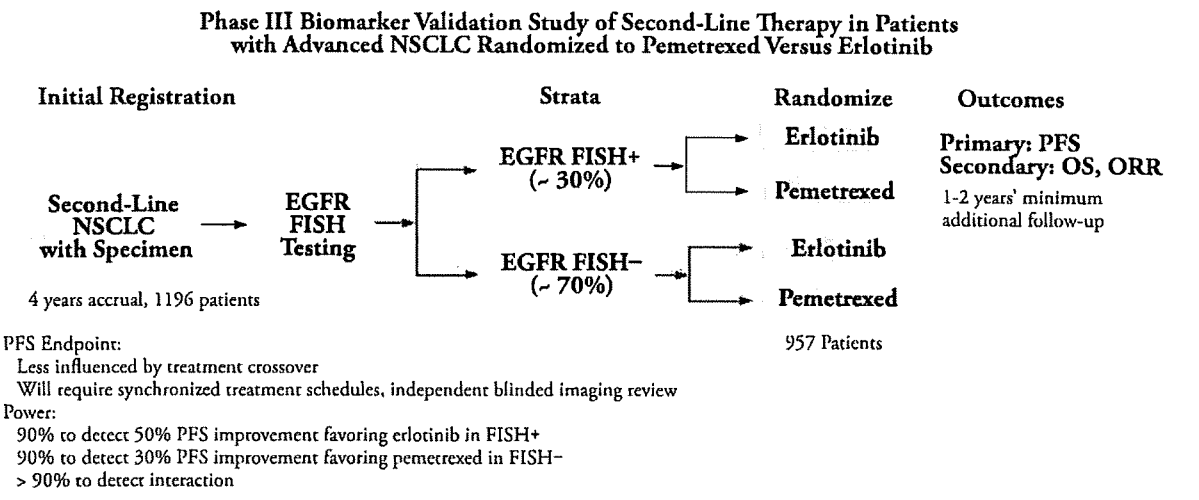
The EORTC is also planning a trial in advanced-stage NSCLC looking at the combination of bortezomib and Apo2L/TRAIL ligand (dulanermin), a direct inducer of apoptosis.

Japan Clinical Oncology Group

There are multiple cooperative groups in Japan, including the JCOG, based in Tokyo, which is fully sponsored by the Ministry of Health. The JCOG has an ongoing phase III trial (PC704) for elderly patients with advanced-stage NSCLC who are randomized to receive single-agent docetaxel versus docetaxel and cisplatin. The study aims to enroll 385 patients, with a primary endpoint of OS.

The West Japan Thoracic Oncology Group (WJTOG) recently completed a large phase III trial (WJTOG 0203) of 3 cycles of chemotherapy followed by gefitinib versus an additional 2 cycles of chemotherapy. This trial was presented at the 2008 ASCO meeting and found no OS advantage with gefitinib but a significant benefit in the adenocarcinoma subset.[17] An ongoing randomized phase III trial by the group (WJTOG 3605) randomizes patients to carboplatin/paclitaxel versus carboplatin/S-1, an oral 5-fluorouracil derivative. This trial aims to enroll 600 patients.

Figure 3 NCCTG 0723 (Intergroup Study): Marker Validation of Erlotinib in Lung Cancer (MARVEL)



Abbreviations: EGFR = epidermal growth factor receptor; FISH = fluorescence in situ hybridization; NCCTG = North Central Cancer Treatment Group; NSCLC = non–small-cell lung cancer; ORR = overall response rate; OS = overall survival; PFS = progression-free survival

The North Japan Lung Cancer Study Group, established in 2002, studied carboplatin with 3-weekly or weekly paclitaxel in elderly patients with NSCLC. The North-East Japan Gefitinib Study Group, established in 2004, not surprisingly has multiple trials of gefitinib, predominantly in patients with known activating mutations in EGFR. Ongoing trials within this group include a phase III trial of first-line gefitinib versus carboplatin/paclitaxel for patients with advanced-stage NSCLC with known EGFR activating mutations. A phase II trial limited to elderly patients aged > 75 years with advanced-stage NSCLC and known EGFR activating mutations is also ongoing.

North Central Cancer Treatment Group

The NCCTG, centered at the Mayo Clinic in Minnesota, has participating centers in 30 states and 2 provinces in Canada and includes sites in Puerto Rico. Historically, the NCCTG has focused on phase II studies with novel therapeutic agents and has participated actively in Intergroup protocols. Dr. Mandrekar led an analysis of NCCTG trials looking at progression-free survival (PFS) compared with response as a predictor of OS in advanced-stage NSCLC. The study looked at PFS at 6 months versus best response versus confirmed response all compared with OS at 12 months.[18] They had data from 343 patients in 4 first-line NSCLC trials. In their trials, approximately 65% of patients had progressed by 6 months, with a median time from progression to death of 5 months. This analysis revealed that neither best response nor confirmed response predicted well for survival at 12 months, but PFS at 6 months was a strong predictor for survival at 12 months, with a 78% agreement (HR, 0.44; 95% CI, 0.34-0.58; $P < .0001$).

N0626 is a randomized phase II study of pemetrexed alone or with sorafenib as second-line therapy in patients with advanced-stage NSCLC. There were 3 dose-limiting toxicities in the first 6 patients, all in patients with squamous histology, so the study now excludes those with squamous histology. The accrual goal is 110 patients. N0528 is a randomized phase II first-line trial of gemcitabine and carboplatin with or without cediranib (AZD2171), a VEGFR TKI. Accrual goal is just under 100 patients, using a dose of cediranib of 30 mg.

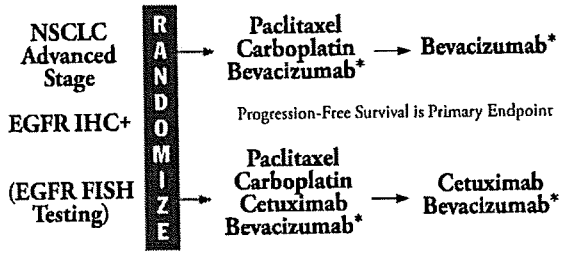
The largest NCCTG trial under development is N0723, also known as the MARker Validation of Erlotinib in Lung cancer (MARVEL) study (Figure 3). This phase III biomarker validation study randomizes patients with advanced NSCLC to pemetrexed versus erlotinib as second-line therapy and will enroll nearly 1200 patients and will analyze all patients for EGFR gene copy number by FISH analysis. In addition to the primary clinical endpoint, the study is unique in engaging all of the North American cooperative groups within the correlative science objectives, a model that will facilitate active support and participation. N0724 is a phase II study for patients with oligometastatic disease who will received standard 4 cycles of platinum-based chemotherapy and then will be randomized to received observation or radiation to known sites of disease. Follow-up will be every 12 weeks after completion of therapy.

N0821 is a planned phase II study of pemetrexed, carboplatin, and bevacizumab in patients with advanced nonsquamous NSCLC aged ≥ 70 years and with a PS of 0/1. This trial is building on encouraging data with the combination presented by Jyoti Patel, MD, at the 2008 ASCO meeting.[19]

National Cancer Institute of Canada Clinical Trials Group

The National Cancer Institute of Canada Clinical Trials Group has been instrumental in development of second-line therapy for patients with advanced-stage NSCLC, most recently erlotinib. BR.21 was a randomized double-blind placebo controlled trial in patients with previously treated NSCLC who had received 1 or 2 previous chemotherapy regimens and received erlotinib or placebo.[9] Interestingly, patients with ECOG PS of 0-3 were eligible. The re-

Figure 4 SWOG 0819: Proposed Phase III Trial of Chemotherapy with or Without Cetuximab (plus Bevacizumab as Eligible)



1236 screened
989 enrolled
618 FISH+

PC = Paclitaxel 200 mg/m² and Carboplatin AUC = 6;
Cetuximab = 400 mg/m², then 250 mg/m² weekly

*In Bevacizumab Eligible: 15 mg/kg every 3 weeks (piloted in S0536)

Correlative Science: Tumor: EGFR/HER pathways; K-*ras*
Genomic DNA: EGFR polymorphisms
Plasma: Proteomic predictor

Abbreviations: AUC = area under the curve; EGFR = epidermal growth factor receptor; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; NSCLC = non–small-cell lung cancer; SWOG = Southwest Oncology Group

sults from this trial, with a response rate of 8.9% with erlotinib and a 2-month improvement in OS (6.7 months vs. 4.7 months; HR, 0.7; $P = .001$), led to approval of the drug in North America.

First-line advanced-stage NSCLC efforts of the NCIC-CTG thoracic group have been focused on cediranib, a VEGFR TKI. BR.24 was a phase II/III trial of first-line carboplatin/paclitaxel with or without cediranib that was recently closed at interim analysis. The study has yet to be presented in its entirety, but it is known that the study arm did meet its efficacy endpoint, but because of toxicity issues, the trial will not continue to phase III. This is despite a dose reduction in cediranib to 30 mg daily (reduced from 45 mg).

The predominant second-line effort of the NCIC-CTG thoracic committee will be participation in N0723 (MARVEL), described above in the NCCTG section. This trial will be known as BRC.3.

Correlative studies are also an important part of NCIC-CTG efforts, with tumor banks for many trials and genomic DNA, urine banks and plasma banks collected as part of BR.24.

Radiation Therapy Oncology Group

The RTOG focuses on radiation questions, so efforts in metastatic disease are limited, but they have played a critical role in better defining therapy for brain metastases in this stage of disease. An ongoing effort in this area is RTOG 0320, a trial for patients with brain metastases from NSCLC. Eligible patients have 1-3 brain metastases ≤ 4 cm in size and not involving the brainstem and no actively progressing extracranial disease for ≥ 1 month. Patients are stratified by age, extracranial cancer, number of metastases (1 versus 2/3) and are randomized to whole-brain radiation (WBRT) plus stereotactic radiosurgery (SRS), WBRT plus SRS and temozolomide (daily for 21 days during WBRT, then continued at the discretion of the investigator), or WBRT plus SRS plus erlotinib at 150 mg orally daily starting with day 1 of WBRT and continuing for ≤ 6 months at the discretion of the investigator. As of June 2008, a total of 80 of a planned 381 patients had been enrolled.

Southwest Oncology Group

Southwest Oncology Group played a major role in establishing carboplatin and paclitaxel as a standard US regimen in S9509, a first-line phase III trial in advanced NSCLC randomizing patients to the then-SWOG standard of cisplatin/vinorelbine versus carboplatin/paclitaxel. Although efficacy was similar, tolerability, as defined by dose delivery and discontinuance of therapy because of toxicity, both favored carboplatin/paclitaxel.[20] Based on these data, SWOG has used carboplatin/paclitaxel as the chemotherapy platform upon which to build targeted-agent chemotherapy regimens in its subsequent trials. S0003, a phase III trial of chemotherapy with or without the hypoxic cytotoxin tirapazamine, found no benefit to the addition of tirapazamine but, in collaboration with Japanese cooperative groups, provided a prospectively designed database for the "common arm" approach, comparing the toxicity, efficacy, and pharmacogenomics of this regimen in S0003 with a common carboplatin/paclitaxel arm in 2 Japanese phase III trials.

A recent SWOG randomized phase II trial (S0342) studied carboplatin/paclitaxel chemotherapy in combination with cetuximab given concurrently or sequentially in advanced-stage NSCLC. Cetuximab is an antibody to EGFR and competitively blocks the binding of EGF and other ligands to EGFR. Two previous phase III trials (Study 099 and FLEX) combining the drug with first-line doublet chemotherapy for NSCLC have now been completed. Study 099, which used no EGFR selection criteria for study entry, demonstrated no significant improvement in response or PFS when the agent was combined with carboplatin/taxane (paclitaxel or docetaxel).[21] FLEX, which combined cetuximab with cisplatin and vinorelbine, found a statistically significant improvement in OS and time to progression but no improvement in PFS with the addition of cetuximab.[22]

A prospectively planned correlative science study incorporated into SWOG 0342 looked at EGFR gene copy number by FISH. This analysis found a strong correlation between FISH positivity and response, PFS, and OS with cetuximab, especially in the concurrent chemotherapy and cetuximab arm.[23] In contrast, in an analysis performed by Dr. Hirsch's group on the TRIBUTE trial of chemotherapy with or without erlotinib, the response was lower in patients with EGFR FISH positivity who received erlotinib/chemotherapy versus placebo/chemotherapy. These contrasting results suggest that, in NSCLC, EGFR TKIs are quite different in terms of interaction with chemotherapy. Building from there, and the encouraging E4599 bevacizumab data, SWOG S0536 subsequently tested a 4-drug regimen of carboplatin/paclitaxel/cetuximab/bevacizumab. Preliminary results suggest that this 4-drug regimen results in encouraging PFS and OS. Taken together, these results led to the development of S0819, a phase III trial that will randomize patients to carboplatin/paclitaxel with or without cetuximab and with bevacizumab for patients eligible for bevacizumab per E4599 entry criteria (Figure 4). After completion of chemotherapy, patients in the cetuximab arm will receive maintenance cetuximab (plus bevacizumab if they were bevacizumab eligible). The study will be statistically powered to validate the role of EGFR FISH as a predictive biomarker, enrolling > 1500 patients in order to accrue

the requisite number who are FISH positive. Besides EGFR FISH, correlative studies will include tumor analysis for EGFR/HER pathway members and K-*ras*, as well as genomic DNA for EGFR polymorphisms and serum analysis of potential proteomic predictors for anti-EGFR and antiangiogenic therapy.

S0709 is a phase II trial of erlotinib versus erlotinib plus chemotherapy in NSCLC patients with PS of 2 and serum proteomics predictive of erlotinib benefit.[15] In the chemotherapy-plus-erlotinib arm, patients will receive carboplatin/paclitaxel on day 1 then erlotinib on days 2-16 of each 21-day cycle to allow for "pharmacodynamic separation," based on earlier data.[24]

Conatumumab (AMG 655) is a proapoptotic agent that directly activates DR-2, leading to the activation of caspases and direct triggering of apoptosis. S0810 will enroll 60 patients per arm and after a run in phase I study to determine the dose of pemetrexed plus conatumumab (5 mg/kg then 15 mg/kg of conatumumab plus full-dose pemetrexed), patients will be randomized to conatumumab alone at 15 mg/kg every 3 weeks or the same dose plus pemetrexed at 500 mg/m^2.

Conclusion

Cooperative groups focused on lung cancer research around the world are a critical component in the fight against this deadly disease. The first successes with targeted agents including bevacizumab and erlotinib have come from these groups. Recent discoveries in the molecular biology of lung cancer are being made by and translated into clinical research through cooperative group efforts, as highlighted by the emerging story of EGFR FISH in the prediction of benefit from erlotinib and cetuximab. Many promising agents and regimens are currently under investigation within the cooperative group system, and the future holds great promise.

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SNP Communication

Twenty Novel Genetic Variations and Haplotype Structures of the *DCK* Gene Encoding Human Deoxycytidine Kinase (dCK)

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

Summary: Deoxycytidine kinase (dCK) is a rate-limiting enzyme in the activation of nucleoside anticancer drugs, such as gemcitabine and cytarabine (Ara-C), to their active metabolites. In this study, the 5′-flanking region, 7 exons and their flanking introns of DCK were comprehensively screened for genetic variations in 256 Japanese cancer patients administered gemcitabine. Twenty-nine genetic variations, including twenty novel ones, were found: 11 in the 5′-flanking region, 1 in the 5′-untranslated region (UTR), 1 in the coding exon, 9 in the 3′-UTR, and 7 in the introns. The novel variations included -1110C>T, -757G>A, -639C>T, -465G>A, -402T>C, -224C>A, -199C>G, IVS1+38G>T, IVS2+78_+83delTTTTTC, IVS3-9C>T, IVS4+12T>C, IVS5+39T>C, 1357A>G, 1545A>T, 1572delA, 1736G>A, 1749G>A, 1838T>C, 1889G>A, and 2048A>T. The frequencies were 0.01 for IVS2+78_+83delTTTTTC, 0.008 for -402T>C, 0.006 for -639C>T and IVS4+12T>C, 0.004 for -757G>A and 1572delA, and 0.002 for the other 14 variations. A known nonsynonymous SNP 364C>T (Pro122Ser) was detected at a 0.061 frequency. Using the detected polymorphisms, linkage disequilibrium analysis was performed, and 24 haplotypes were identified or inferred. Our findings suggest considerable ethnic differences in genetic variations of *DCK* and provide fundamental and useful information for genotyping *DCK* in the Japanese and probably other Asian populations.

Keywords: *DCK*, genetic variation, haplotype, gemcitabine, ethnic differences

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On February 15, 2008, these variations were not found in "A database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/)", "dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/)", or "PharmGKB (http://www.pharmgkb.org/do/)".
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Introduction

Deoxycytidine kinase (EC 2.7.1.74, dCK) is a key enzyme in the salvage pathway of deoxyribonucleotide biosynthesis and is responsible for phosphorylation of both pyrimidine and purine deoxyribonucleosides to the corresponding deoxyribonucleotides.[1] dCK also catalyzes the rate-limiting step in the phosphorylation of pharmacologically important anticancer and antiviral drugs such as 2′,2′-difluorodeoxycytidine (gemcitabine), cytosine arabinoside (ara-C), 2-chlorodeoxyadenosine (cladribine), and 2′,3′-dideoxycytidine.[2]

The *DCK* gene consists of 7 exons and spans over 34-kb on chromosome 4 (4q13.3-q21.1).[3] The transcription regulatory region of *DCK* is GC-rich and lacks a TATA-box but harbors a number of binding sites for transcription factors such as Sp1 and E2F.[4-6] Human dCK protein (260 amino acid residues) is constitutively expressed throughout the cell cycle and is present at low levels in most tissues. Therefore, this enzyme could be involved in the activation of chemotherapeutic nucleoside analogues in tumor cells as well as normal cells.[7-9] Expression levels of dCK are critical determinants of gemcitabine and ara-C antitumor activities, and dCK-deficient cells are highly resistant to nucleoside analogues.[10] Furthermore, the introduction of *DCK* cDNA into dCK-deficient tumor cell lines restores *in vitro* sensitivities to ara-C,[11,12] and the decreased dCK expression is known to be associated with *in vitro* acquired resistance to gemcitabine.[13]

Recently, several single nucleotide polymorphisms (SNPs) and haplotypes of *DCK*, including four nonsynonymous SNPs, 70A>G (Ile24Val), 356C>G (Ala119Gly), 364C>T (Pro122Ser) and 727A>C (Lys243Gln), have been identified in Africans, Europeans, and Chinese[14-16] and published in the PharmGKB database. *In vitro* functional characterization showed that three nonsynonymous variations, 70A>G (Ile24Val), 356C>G (Ala119Gly) and 364C>T (Pro122Ser), were associated with dCK activity and expression.[16] In Chinese, several SNPs were detected in the promoter region, and the haplotype with two regulatory SNPs, −360C>G and −201C>T, was associated with increased transcriptional activity.[14] However, there has been no report of a *DCK* SNP survey and haplotype analysis for a Japanese population. In this study, all 7 exons and their surrounding introns were resequenced for comprehensive screening of *DCK* genetic variations. Sequence analysis detected 29 variations from 256 Japanese cancer patients administered gemcitabine. Frequencies of haplotypes with both regulatory SNPs, −360C>G and −201C>T, and nonsynonymous SNP 364C>T (Pro122Ser) were estimated in a Japanese population, and ethnic differences among Japanese, Chinese, Europeans and Africans were shown.

Materials and Methods

Human genomic DNA samples: All 256 Japanese cancer patients were administered gemcitabine at the National Cancer Center Hospital and National Cancer Center Hospital East. Total DNA was extracted from blood leukocytes and used as template in the polymerase chain reaction (PCR). The ethical review board of the National Cancer Center and National Institute of Health Sciences approved this study. Written informed consent was obtained from all participants.

PCR conditions for DNA sequencing: The Genbank reference sequence NT_006216.14 was used for primer design and SNP detection. First, the entire *DCK* gene was divided into two regions (exons 1 and 2 and exons 3 to 7), and each region was amplified from 100 ng of genomic DNA using 1.25 units of *Z-Taq* (Takara Bio. Inc., Shiga, Japan) with 0.2 μM primers listed in **Table 1** (1st PCR). The first PCR conditions consisted of 30 cycles of 98°C for 5 sec, 60°C for 10 sec, and 72°C for 150 sec. Next, each exon except for exon 1 was amplified by *Ex Taq* (1.25 units) (Takara Bio. Inc.) with appropriate primers (0.5 μM) designed in the introns (**Table 1**, 2nd PCR). Conditions of the second round PCR with *Ex Taq* were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. For amplification of the region from 1.5-kb upstream of the translation initiation site to exon 1, *LA Taq* (2.5 units) (Takara Bio. Inc.) with GC buffer I and exon 1 specific primers (0.5 μM) were used. PCR with *LA Taq* was carried out under the following conditions: 94°C for 1 min followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 3 min, and then a final extension at 72°C for 5 min. Following the PCR, products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in **Table 1** (Sequencing). Excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All variations were confirmed by sequence analysis of PCR products generated by a new amplification of the original genomic DNA templates. Furthermore, the rare SNPs found in only one sample as heterozygotes were confirmed by re-sequencing the PCR fragments produced by amplification with a high fidelity DNA polymerase KOD-Plus- (TOYOBO, Tokyo, Japan).

Haplotype analysis: Hardy-Weinberg equilibrium and linkage disequilibrium (LD) analyses were performed by SNPAlyze software (Ver 3.1, Dynacom Co., Yokohama, Japan). All allele frequencies were in Hardy-Weinberg equilibrium. Some haplotypes were unambiguously

Table 1. Primer sequences used in this study

	Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified region[a]	Length (bp)
1st PCR	Exons 1 to 2	CGGTTTATTAGTGTTACTGGATGGG	AGTCACCCCTCAGTAACCTCAAGAA	364945_371347	6403
	Exons 3 to 7	GGCATGGTACTGCTTGGTTTTTTCA	TCTAGGTGGCTCGGTATAAGTTTCA	394431_403862	9432
2nd PCR	Exon 1	ACCAAGTGCTTCAAGAGTCC	AAAGAGGGAGCAGAGGTTCA	365110_366917	1808
	Exon 2	GCAGGGAGCCTTTTCATTTT	GATATGGAGAGCCAACTGTA	370620_371078	459
	Exon 3	AGGATTTTCCAGACCTCAGA	ACCAGACTGCTGAGGGATTT	394902_395443	542
	Exon 4	TCTGCTTTCCACGGCACTAT	ATTGAGGAAGCACAAAGAGC	396162_396663	502
	Exon 5	CAAGTGGCTGAAAAGCTCAT	ACGCTATCAATACCACCAAG	398368_398883	516
	Exon 6	GACAACATTTTGATTTTCCAAG	GGATCTTTATTTTAGCTCAGGC	399289_399681	393
	Exon 7 fragment 1	TGGCATTGTGGTAGTTACTT	ATACACAGGAAAAACACTGC	401952_403074	1123
	Exon 7 fragment 2	AGATGGTTCCAGTATCAGCA	TCCAGACCACCATTAGGCTC	402353_403692	1340
Sequencing	Exon 1	AGTGCTTCAAGAGTCCCAAT	GATGGGACAAATGCAGTGTA		
		TGCTGTTCTTTTGCTTATGC	CTGAGAGGCTGCTTGTTCCA		
		TCTCAGTGCCTGTTTTCCCA	AAAACCCGCCTCTCTAGTGG		
		CACTAGAGAGGCGGGTTTTC	GGTGTCGGGGTTTGACTTTG		
		GCAGGTCAGGATCTGGCTTA	AGGTAAGGGAAGGATGCTCT		
	Exon 2	GCAGGGAGCCTTTTCATTTT	GATATGGAGAGCCAACTGTA		
	Exon 3	AGGATTTTCCAGACCTCAGA	AAGTCCAGTTCTAAGATAAAAAC		
	Exon 4	TGAAATGATACATGTGTTGATG	ATTGAGGAAGCACAAAGAGC		
	Exon 5	CAAGTGGCTGAAAAGCTCAT	GAAGATACCAATAAGCAAAACG		
	Exon 6	TTGTTGAATTTCTGATTATTTTA	GGATCTTTATTTTAGCTCAGGC		
	Exon 7 fragment 1	TGGCATTGTGGTAGTTACTT	AAAACGATTAAAAACTTGGGTT		
		AGATGGTTCCAGTATCAGCA	GACTTTAACTTTATAGCAGGCT		
	Exon 7 fragment 2	GCTTTCTCTACTGTCTGGAT	ATACACAGGAAAAACACTGC		
		TTTGTTTAGTTAAGGTGTGC	ATTATGACCACCACACTGAG		

[a]The reference sequence is NT_006216.14.

assigned in subjects with homozygous variations at all sites or a heterozygous variation at only one site. Separately, diplotype configurations (combinations of haplotypes) were inferred by LDSUPPORT software, which determines the posterior probability distribution of the diplotype configuration for each subject based on estimated haplotype frequencies.[17] The haplotypes are described as numbers plus small alphabetical letters.

Results and Discussion

The *DCK* 5′-flanking region (up to 1.5-kb upstream of the translational start site), all 7 exons and their flanking introns were sequenced in 256 Japanese cancer patients administered gemcitabine, and 29 variations, including 20 novel ones were found (see **Table 2**). The novel variations were −1110C>T, −757G>A, −639C>T, −465G>A, −402T>C, −224C>A and −199C>G in the 5′-flanking region (A of the translational start codon is numbered +1), IVS1+38G>T in intron 1, IVS2+78_+83delTTTTTC in intron 2, IVS3−9C>T in intron 3, IVS4+12T>C in intron 4, IVS5+39T>C in intron 5, and 1357A>G, 1545A>T, 1572delA, 1736G>A, 1749G>A, 1838T>C, 1889G>A, and 2048A>T in the 3′-noncoding region of exon 7. The frequencies were 0.01 for IVS2+78_+83delTTTTTC, 0.008 for −402T>C, 0.006 for −639C>T and IVS4+12T>C, 0.004 for −757G>A and 1572delA and 0.002 for the other 14 variations.

Nine SNPs detected in this study were previously reported[14–16] and/or found in the dbSNP and PharmGKB databases. Among them, two regulatory SNPs, −360C>G and −201C>T, were found at allele frequencies of 0.131, which are comparable to those in a Chinese population (0.156),[14] but higher than those in Europeans (0.01–0.025) and Africans (not detected).[15,16] The frequency of nonsynonymous SNP 364C>T (Pro122Ser) in Japanese (0.061) is slightly higher than that in Europeans (0.015–0.025) and Africans (0.017).[15,16] Other nonsynonymous SNPs, 70A>G (Ile24Val), 356C>G (Ala119Gly) and 727A>C (Lys243Gln), found in Europeans and Africans were not detected in a Japanese population.

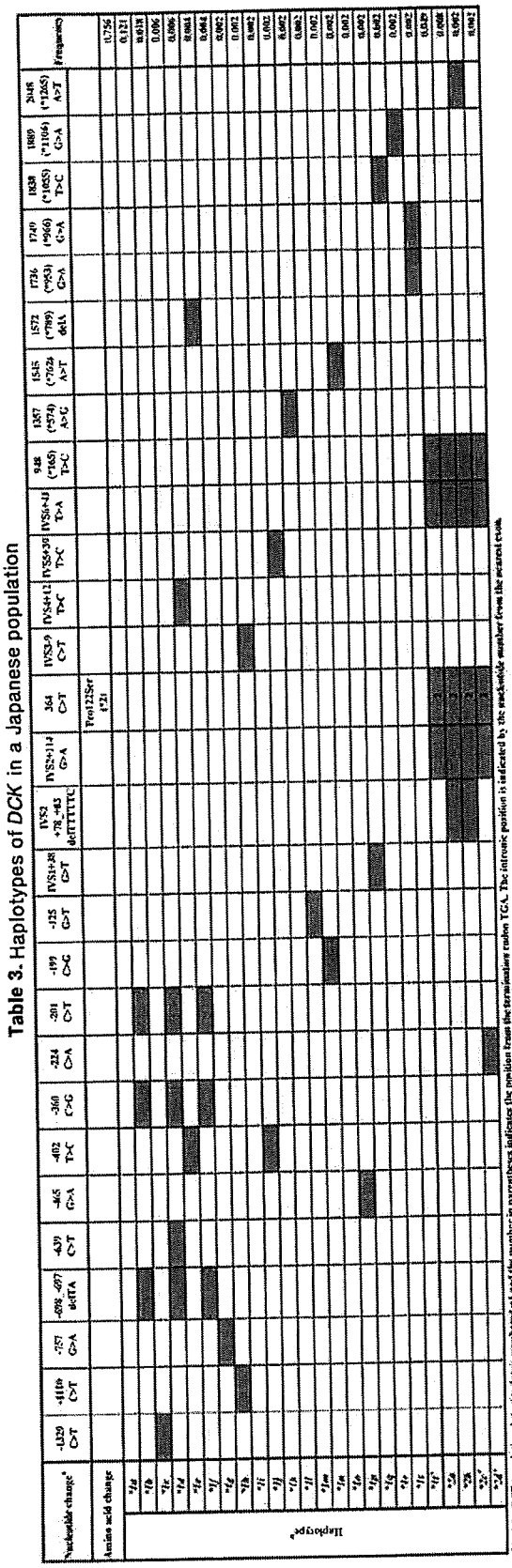
The 5′-flanking region of the *DCK* gene contains binding sites for several transcription factors which regulate *DCK* expression.[4–6] In this study, 11 variations were found in the *DCK* 5′-flanking region. Among them, two associated SNPs, −360C>G and −201C>T, were reported to increase ara-C efficacy: −360C>G results in

Table 2. Genetic variations of *DCK* found in a Japanese population

SNP ID		Reference	Location	Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Allele frequency
This study	NCBI (dbSNP)			NT_006216.14	From the translational initiation site or from the nearest exon			
MPJ6_DCK001	rs1906021		5′-Flanking	365234	−1329[c]	AGGATTGGCCTGC/TGACCAATCAGAG		0.018
MPJ6_DCK002[a]			5′-Flanking	365453	−1110[c]	ACTAAAAATGCAC/TTCATCTAGCTGG		0.002
MPJ6_DCK003[a]			5′-Flanking	365806	−757[c]	TCCCACTGGCAGG/ATAAATGGGCTAA		0.004
MPJ6_DCK004			5′-Flanking	365865_365866	−698_−697[c]	ATGAAAAGCACATA/-GAAGAAAACAGC		0.131
MPJ6_DCK005[a]			5′-Flanking	365924	−639[c]	GACGGCACTTCGC/TCTGATAGTCTTC		0.006
MPJ6_DCK006[a]			5′-Flanking	366098	−465[c]	AAAGCCTGGCACG/AGCCCACTGCAGG		0.002
MPJ6_DCK007[a]			5′-Flanking	366161	−402[c]	GTCCACCCTTCCT/CCCCCACCCGACT		0.008
MPJ6_DCK008		13, 14, 15	5′-Flanking	366203	−360[c]	GCCCTGCCCGGGC/GGCCTGGCTGCTT		0.131
MPJ6_DCK009[a]			5′-Flanking	366339	−224[c]	AGCTAGGGAGCGC/AGGCTTGAGGAGG		0.002
MPJ6_DCK010	rs2306744	13, 14, 15	5′-Flanking	366362	−201[c]	GGGCGGGGCCGCC/TCCGCAGGCCCGC		0.131
MPJ6_DCK011[a]			5′-Flanking	366364	−199[c]	GCGGGGCCGCCCC/GGCAGGCCCGCCA		0.002
MPJ6_DCK012		13	Exon 1 (5′-UTR)	366438	−125[c]	GCGGGCCGGTGAG/TCTCACTAGCTGA		0.002
MPJ6_DCK013[a]			Intron 1	366691	IVS1+38	CGCAAGGCTGGGG/TTGTCGCGGCAGT		0.002
MPJ6_DCK014[a]			Intron 2	370987_370992	IVS2+78_+83	TTTTTCTTTTTCTTTTTC/-AATAAAACTTTC		0.010
MPJ6_DCK015	rs6446988	15	Intron 2	371023[b]	IVS2+114	CGCTTTAGGTATG/ATATCTTCATCTA		0.061
MPJ6_DCK016		14, 15	Exon 3	395250	364[c]	GATGCAGAGAAAC/TCTGTATTATTTT	Pro122Ser	0.061
MPJ6_DCK017[a]			Intron 3	396277	IVS3−9	TTGATGAAGACTC/TTCTTTTAGGTAT		0.002
MPJ6_DCK018[a]			Intron 4	396445	IVS4+12	GGTAAAACCCAAT/CAAAAATGTGTTT		0.006
MPJ6_DCK019[a]			Intron 5	398697	IVS5+39	ATTTTAAATACCT/CTTGTTACCTTTG		0.002
MPJ6_DCK020	rs1486271	14	Intron 6	399523[b]	IVS6+41	TTTGTTTTTTCTT/AAAAAAGTGTACT		0.061
MPJ6_DCK021	rs4643786	15	Exon 7 (3′-UTR)	402270[b]	948(*165)[c]	AAAACTTTTTGAT/CCAGTTTCTTTTC		0.061
MPJ6_DCK022[a]			Exon 7 (3′-UTR)	402679	1357(*574)[c]	TCTGCTTTCTCTA/GCTGTCTGGATTA		0.002
MPJ6_DCK023[a]			Exon 7 (3′-UTR)	402867	1545(*762)[c]	TATCCTGAAAGCA/TTTATTTTTTGTT		0.002
MPJ6_DCK024[a]			Exon 7 (3′-UTR)	402894	1572(*789)[c]	ATAGGAAATAAAA/-TTAATGAAGACA		0.004
MPJ6_DCK025[a]			Exon 7 (3′-UTR)	403058	1736(*953)[c]	TTAAGGTGTGCAG/ATGTTTTTCCTGT		0.002
MPJ6_DCK026[a]			Exon 7 (3′-UTR)	403071	1749(*966)[c]	TGTTTTTCCTGTG/ATATTAAACCTTT		0.002
MPJ6_DCK027[a]			Exon 7 (3′-UTR)	403160	1838(*1055)[c]	AACTACTATTTTT/CCTTTCCAAGTCA		0.002
MPJ6_DCK028[a]			Exon 7 (3′-UTR)	403211	1889(*1106)[c]	GATGATAATTTAG/ATGGATTAACCAG		0.002
MPJ6_DCK029[a]			Exon 7 (3′-UTR)	403370	2048(*1265)[c]	TCTTAAGTATAAA/TCCTTATGAACTA		0.002

[a]Novel variations detected in this study.
[b]The reference sequence NT_006216.14 has the minor allele.
[c]A of the translation initiation codon ATG is numbered +1 and the number in the parentheses indicates the position from the termination codon TGA.

Table 3. Haplotypes of *DCK* in a Japanese population



*A of the *DCK* translational start codon is numbered +1 and the number in parentheses indicates the position from the terminator codon TGA. The intronic position is indicated by the nucleotide number from the nearest exon.

*White cell, major allele; gray cell, minor allele. The haplotypes are described as numbers plus letters.

*Ambiguous haplotype inferred as only one chromosome.

a novel AP2 site, and −201C>T leads to loss of Sp1 and AP2 sites.[14] Biological significance of the other 9 variations in the 5′-flanking region should be further determined.

Using the detected variations, linkage disequilibrium (LD) analysis was performed. A perfect linkage ($r^2 = 1$) was observed among −698_−697delTA, −360C>G and −201C>T, and among IVS2+114G>A, 364C>T, IVS6+41T>A and 948T>C. Therefore, the entire region was analyzed as one LD block for haplotype estimation. The determined/inferred haplotypes are shown as numbers plus small alphabetical letters (**Table 3**). In this study, the haplotypes without amino acid changes were defined as the **1* group, and the haplotype harboring the nonsynonymous SNP (Pro122Ser) was assigned as the **2* group. Several haplotypes were first unambiguously assigned by homozygous variations at all sites (**1a*, **1b* and **2a*) or heterozygous variation at only one site (**1c* and **1g* to **1s*). Separately, the diplotype configurations (combinations of haplotypes) were inferred by LDSUPPORT software. The additionally inferred haplotypes were three **1* subtypes (**1d* to **1f*), and **2b*. The most frequent haplotype was **1a* (frequency, 0.756), followed by **1b* (0.121), **2a* (0.049), and **1c* (0.018). The frequencies of the other minor haplotypes were less than 0.01. Some haplotypes (**1t*, **2c* and **2d*) were inferred in only one subject and ambiguous (**Table 3**).

Previously, Shi *et al.*[14] reported that the two SNPs, −360C>G and −201C>T, were in perfect LD. It was reported that these SNPs were associated with the clinical outcome for Chinese AML patients treated with ara-C, which was explained by increased transcriptional activity.[14] These SNPs were also found in Europeans at a low frequency (0.025), but their association with *DCK* mRNA expression levels was not confirmed. In our study, haplotypes harboring these SNPs were **1b* (frequency, 0.121), **1d* (0.006) and **1f* (0.004). It must be noted that −698_−697delTA is completely associated with these haplotypes.

Lamba *et al.*[16] reported that the recombinant 122Ser dCK protein showed reduced enzyme activity (43±4% of the wild-type), and lymphoblast cell lines from subjects carrying heterozygous 364C>T had reduced dCK activity compared with those from homozygous wild-type subjects.[16] In our study, the haplotype frequency of the **2* group harboring SNP 364C>T was 0.061. The **2* group also harbors the known SNPs, IVS2+114G>A, IVS6+41T>A and 948T>C. These three SNPs were also found in Europeans and Africans at different frequencies (0.05 in Europeans and 0.767 in Africans) and constitute the common haplotype group, Group 1/Block 1.[16] However, Group 1 haplotype did not harbor 364C>T in both populations. The LD profile of Japanese was similar to that of Europeans except for the linkage of 364C>T, but different from that of Africans. In a Chinese popula-

tion (n=48), 364C>T (Pro122Ser) and three SNPs, IVS2+114G>A, IVS6+41T>A and 948T>C, were not found.[14] Thus, these findings indicate considerable ethnic differences in *DCK* SNPs and haplotypes.

In conclusion, 29 variations including 20 novel ones were identified in *DCK* from 256 Japanese cancer patients administered gemcitabine. Using the detected polymorphisms, 24 haplotypes were determined or inferred. Our findings suggest considerable ethnic differences in genetic variations of *DCK* and provide fundamental and useful information for genotyping *DCK* in the Japanese and probably other Asian populations.

Acknowledgments: We thank Ms. Chie Sudo for her secretarial assistance.

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Performance Status and Sensitivity to First-line Chemotherapy Are Significant Prognostic Factors in Patients With Recurrent Small Cell Lung Cancer Receiving Second-line Chemotherapy

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BACKGROUND. To the authors' knowledge, the prognostic factors in recurrent small cell lung cancer (SCLC) patients treated with second-line chemotherapy have not yet been clearly identified to date.

METHODS. Between July 1992 and December 2003, 232 of 515 patients who were diagnosed to have SCLC at the National Cancer Center Hospital East were administered second-line chemotherapy for recurrent disease. The authors retrospectively analyzed the relation between clinical factors evaluated at the time of recurrence and the response to second-line chemotherapy or survival in these patients.

RESULTS. The results of univariate analyses revealed that response was significantly associated with the performance status (PS) alone, whereas survival was significantly associated with the PS, disease extent, and sensitivity to first-line chemotherapy. Multivariate analysis identified PS ($P < .0001$) and sensitivity to first-line chemotherapy ($P = .0024$) as the independent prognostic factors for survival. When the patients were grouped according to these 2 significant prognostic factors, the survival of patients with a PS of 0 to 1 was significantly better than that of the patients with a PS of 2 to 4 both among cases that were sensitive and those that were refractory to first-line chemotherapy. Although the survival of sensitive recurrent cases was significantly better than that of the refractory recurrent cases among the patients with a PS of 0 to 1 patients, no survival difference was observed between the sensitive and refractory recurrent cases in the patients with a PS of 2 to 4.

CONCLUSIONS. Both PS and sensitivity to initial chemotherapy were found to be significant prognostic factors for survival in recurrent SCLC patients treated with second-line chemotherapy. These 2 factors should therefore be used as stratification factors in future clinical trials. *Cancer* **2008;113:2518–23.** © *2008 American Cancer Society.*

KEYWORDS: small cell lung cancer, second-line chemotherapy, prognostic factor, performance status, sensitive recurrence, refractory recurrence.

Although the proportion of small cell lung cancer (SCLC) among cases of lung cancer has been decreasing in recent years, it still accounts for 14% of all new lung cancer cases, and the actual number of patients was estimated to be 77,000 in the US and Europe in 2004.[1] In general, SCLC is an exceedingly aggressive cancer, and greater than 66% of patients have clinically obvious metastatic disease at the time of diagnosis.[2] SCLC is also extremely sensitive to chemotherapy; therefore, the main treatment strategy for SCLC is

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systemic chemotherapy. Currently, both cisplatin plus etoposide (PE) and cisplatin plus irinotecan (IP) are considered as standard chemotherapeutic regimens for SCLC.[3,4] Despite the high initial sensitivity to chemotherapy, the majority of patients develop disease recurrence. The prognosis of patients with recurrent SCLC is usually abysmal, and the overall survival time after recurrence is reportedly 2 to 4 months.[5]

In general, second-line chemotherapy is considered for cases with recurrent SCLC, and a few studies have reported on the efficacy of some second-line treatments.[6,7] For example, a prospective randomized trial comparing oral topotecan with best supportive care (BSC) revealed the benefits of treatment with oral topotecan in terms of the survival and quality of life.[7]

Although some studies have shown the importance of both response and the duration of the response to initial chemotherapy in predicting the efficacy of second-line chemotherapy,[8-10] the number of studies conducted to identify the prognostic factors in recurrent SCLC patients is quite limited. In this retrospective study, we investigated the prognostic factors in recurrent SCLC patients administered second-line chemotherapy to determine the factors that need to be used for stratifying the patients in future clinical trials.

MATERIALS AND METHODS

Patient Flow

Between July 1992 and December 2003, 515 patients were diagnosed to have SCLC at the National Cancer Center Hospital East, and 474 of these patients received initial chemotherapy with or without thoracic radiotherapy. Of 474 patients, radiographic response was observed in 409 patients, with 98 demonstrating complete response and 311 demonstrating partial response. An evaluation in April 2007 revealed that among these responders, 322 had developed disease recurrence, 75 had maintained responses, and 12 patients could not be evaluated for disease recurrence. Thus, 387 patients (including the 322 with disease recurrence and the 65 nonresponders) were considered potential candidates for second-line chemotherapy. Of these, 232 received second-line chemotherapy, whereas the remaining 155 did not. There were no distinct eligibility criteria for second-line chemotherapy, and the decision to administer chemotherapy was based on the patient's general condition and willingness to undergo second-line therapy. The patient flow is shown in Figure 1. Among patients who received second-line chemotherapy, those who deemed to have stable disease or not to be evaluable to first-line chemotherapy were treated right after completion of front-line therapy. All patients' data were obtained from our database.

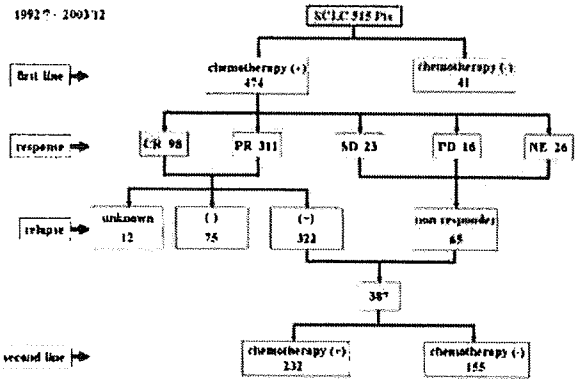

FIGURE 1. Patient flow is depicted. CR indicates complete response; NE, not evaluable; PD, progressive disease; PR, partial response; Pts, patients; SCLC, small-cell lung cancer; SD, stable disease; +, positive; −, negative.

Analyzed Clinical Factors

The correlations between clinical factors evaluated at the time of disease recurrence, such as the age (<70/≥70), sex (women/men), Eastern Cooperative Oncology Group performance status (PS) (0-1 or 2-4), disease extent (limited disease [LD]/extensive disease), sensitivity to first-line chemotherapy (sensitive/refractory), and response to second-line chemotherapy or survival after disease recurrence were retrospectively investigated in the 232 patients. In this study, patients who responded to initial chemotherapy and developed disease recurrence more than 3 months after the completion of chemotherapy were defined as sensitive recurrence cases, whereas patients who did not respond to initial chemotherapy or developed disease recurrence within 3 months were defined as refractory recurrence cases.

Tumor Evaluation and Statistical Analysis

Tumor response was re-evaluated by 2 physicians (Y.H.K. and K.G.) using the Response Evaluation Criteria in Solid Tumors (RECIST).[11] The survival time was measured from the date of disease recurrence. The survival curve was estimated by the Kaplan-Meier method, and compared by the log-rank test. Comparison between each clinical factor and response was performed by the chi-square test. Multivariate analysis was conducted according to the Cox proportional hazard model. $P < .05$ was considered to denote statistical significance. All statistical analyses were performed using StatView statistical

TABLE 1
Characteristics of All Patients at the Time of Disease Recurrence (N = 387)

Characteristics	Second-line Chemotherapy (+) (n=232)	(−) (n=155)	P
Age at recurrence, y			<.0001
Median	65	68	
Range	30-80	28-87	
Gender			.9867
Women	38 (16%)	25 (16%)	
Men	194 (84%)	130 (84%)	
PS at recurrence			<.0001
0-1	162 (70%)	43 (28%)	
2-4	70 (30%)	112 (72%)	
Disease extent at recurrence			.0476
LD	65 (28%)	30 (19%)	
ED	167 (72%)	125 (81%)	
Response to first-line chemotherapy			<.0001
CR/PR	216 (93%)	108 (70%)	
SD/PD	16 (7%)	47 (30%)	
Sensitivity to first-line chemotherapy			.1661
Sensitive	146 (63%)	63 (41%)	
Refractory	86 (37%)	92 (59%)	

+ indicates positive; −, negative; PS, performance status; LD, limited disease; ED, extensive disease; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

software (version 5.0; Abacus Concepts, Berkley, Calif).

RESULTS

Patient Characteristics

The characteristics of the 387 patients who were believed to be potential candidates for second-line chemotherapy (of whom only 232 eventually received second-line chemotherapy, designated as the chemotherapy group) are listed in Table 1. The patients in the chemotherapy group were significantly younger ($P < .0001$), had better PS ($P < .0001$), and had a higher frequency of LD ($P = .0476$) than the nonchemotherapy group. Whereas the response to first-line chemotherapy was significantly different ($P < .0001$), the sensitivity to first-line chemotherapy was not significantly different ($P = .1661$) between the 2 groups, and approximately 33% of the patients who received second-line chemotherapy were refractory recurrence cases. As first-line chemotherapy, 156 patients (67%) had received platinum plus etoposide combination chemotherapy, and 24 (10%) had received the IP regimen. The second-line chemotherapy regimens administered to the 232 patients are listed in Table 2. At our hospital, the vast majority of the patients had received some kind of platinum-based combination chemotherapy, such as cisplatin, vincristine, doxorubicin, and etoposide; cisplatin, etoposide, and irinotecan (PEI); IP; PE; or carboplatin plus etoposide. The distribution of these regimens was similar in the sensitive and refractory recurrence patients.

TABLE 2
Second-line Chemotherapy Regimens Administered to 232 Patients

Regimen	No. of Patients	No. Sensitive (%)	No. Refractory (%)
CODE	80	50 (34)	30 (35)
PEI	44	17 (12)	27 (31)
IP	34	28 (19)	6 (7)
PE	19	13 (9)	6 (7)
CE	14	12 (8)	2 (2)
TOP	14	9 (6)	5 (6)
CPT-11	13	9 (6)	4 (5)
AMR	6	5 (4)	1 (1)
Others	8	3 (2)	5 (6)
Total	232	146 (100)	86 (100)

CODE indicates cisplatin, vincristine, doxorubicin, and etoposide; PEI, cisplatin, etoposide, and irinotecan; IP, cisplatin and irinotecan; PE, cisplatin and etoposide; CE, carboplatin and etoposide; TOP, topotecan; CPT-11, irinotecan; AMR, amrubicin.

TABLE 3
Univariate Analysis for Response and Survival

Characteristics	No. of Patients	Response Rate, %	P	MST, Months	P
Age at recurrence, y					
<70	167	56	.5058	9.0	.6347
≥70	65	62		8.8	
Gender					
Women	38	68	.1826	10.0	.5672
Men	194	55		8.7	
PS at recurrence					
0-1	162	63	.0126	11.0	<.0001
2-4	70	44		4.9	
Disease extent at recurrence					
LD	65	62	.5085	12.6	.0043
ED	167	56		7.3	
Sensitivity to first-line chemotherapy					
Sensitive	146	60	.4413	10.6	.0016
Refractory	86	53		6.8	

MST indicates median survival time; PS, performance status; LD, limited disease; ED, extensive disease.

Predictive and Prognostic Factors

According to the results of the univariate analyses, response was significantly associated with the PS alone, whereas survival was significantly associated with the PS, disease extent, and sensitivity to first-line chemotherapy (Table 3). Survival curves drawn according to the PS and sensitivity to first-line chemotherapy are shown in Figure 2 and 3, respectively. Multivariate analysis identified PS ($P < .0001$) and

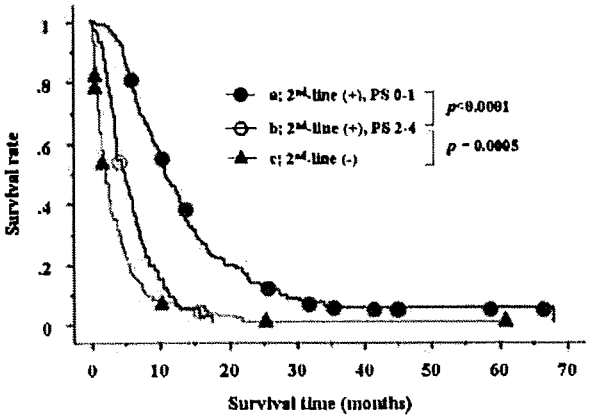

FIGURE 2. Survival curves according to the performance status (PS) at the time of disease recurrence. + indicates positive; −, negative.

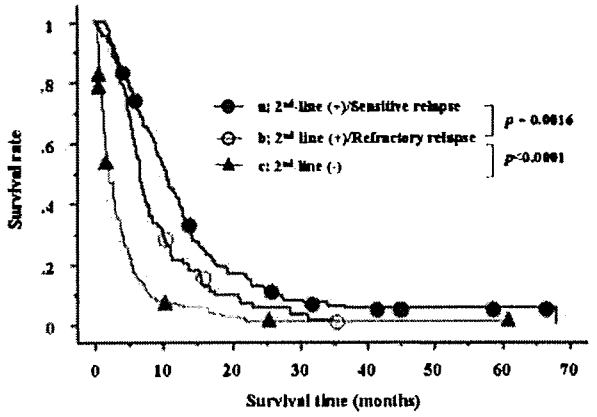

FIGURE 3. Survival curves according to sensitivity to first-line chemotherapy. + indicates positive; −, negative.

sensitivity to first-line chemotherapy ($P = .0024$) as the independent prognostic factors for survival (Table 4). The survival of patients with a PS of 2 to 4 ($P = .005$) (Fig. 2) and refractory disease recurrences ($P < .0001$) (Fig. 3) was significantly better than that of those who did not receive second-line chemotherapy.

In addition, we performed further analysis, in which all patients who received second-line chemotherapy were divided into 4 groups according to the combination of the 2 identified independent prognostic factors for survival: Group A (PS of 0-1/sensitive recurrence), Group B (PS of 0-1/refractory recurrence), Group C (PS of 2-4/sensitive recurrence), and Group D (PS of 2-4/refractory recurrence). The survival curves for each group are shown in Figure 4. The survival of patients with a PS of 0 to 1 was significantly better than that of the patients with a PS of 2 to 4 among both cases with sensitive

TABLE 4
Multivariate Analysis for Survival

Variables	Odds Ratio	95% CI	P
PS at recurrence, 0-1	3.171	2.307-4.357	<.0001
Disease extent at recurrence, LD	1.308	0.956-1.790	.093
Sensitivity to first-line chemotherapy, sensitive	1.544	1.166-2.043	.0024

95% CI indicates 95% confidence interval; PS, performance status; LD, limited disease.

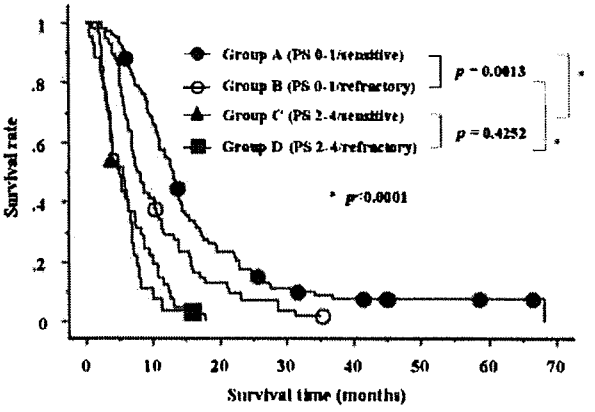

FIGURE 4. Survival curves according to the 2 independent prognostic factors. PS indicates performance status.

(Group A vs Group C; $P < .0001$) and those with refractory recurrence (Group B vs Group D; $P = .0001$). Whereas the survival of the sensitive recurrence cases was significantly better than that of the refractory recurrence cases among the patients with a PS of 0 to 1 (Group A vs Group B; $P = .0013$), no survival difference was observed between the sensitive and refractory recurrence cases among the patients with a PS of 2 to 4 patients (Group C vs Group D; $P = .4252$).

Among the 232 patients who received second-line chemotherapy, 29 received the same regimen as first-line chemotherapy, and the rest received a regimen different from first-line chemotherapy. However, these differences did not appear to have an impact on either response ($P = .7519$) or survival ($P = .5873$).

DISCUSSION

Some studies have shown the importance of both response and the duration of the response to initial chemotherapy in predicting the survival of recurrent SCLC patients receiving second-line chemotherapy,[8-10] and currently it is widely accepted that recurrent SCLC patients should be classified into 2 groups: cases with sensitive recurrence and those with refrac-

tory recurrence.[12] In contrast, Sundstrom et al, who recently analyzed 19 clinical factors at both the time of initial diagnosis and the time of recurrence, have suggested that the PS at the time of disease recurrence, and not the sensitivity status to first-line chemotherapy, was the only significant prognostic indicator for survival after second-line chemotherapy.[13] In this study, we investigated the relation between clinical factors evaluated at the time of disease recurrence and survival after recurrence, and identified both PS and sensitivity to first-line chemotherapy as being significant prognostic factors for survival.

Some may argue that the survival time of the patients with a PS $\geq$3 in this study was too short, which might have strongly influenced the inferior survival of the patients with a PS of 2 to 4 as compared with that of the patients with a PS of 0 to 1. Although our study included 18 cases with a PS $\geq$3 among the patients administered second-line chemotherapy, the results of the analyses were found to be the same even after exclusion of these patients with a PS $\geq$3 (data not shown). This finding suggests that the prognosis of the patients with a PS of 2 is clearly different from that of the patients with a PS of 0 to 1 patients. The diversity of our second-line regimens may be criticized as well, because the differences in the regimens could have affected the patients' outcomes. However, to our knowledge, there are no comparative studies suggesting the superiority of any particular regimen for second-line chemotherapy. At our hospital, as shown in Table 2, mainly platinum-based combination chemotherapy is used even for second-line chemotherapy, and various agents are combined with platinum agents.

The results of the current study indicate that the prognosis of patients with impaired PS is inevitably poor. In such patients, no survival difference was found between the cases with sensitive and those with refractory recurrence. Does this mean that patients with a PS $\geq$2 should not receive second-line chemotherapy? A phase 3 trial comparing oral topotecan with BSC demonstrated a significant survival advantage of oral topotecan, and such survival benefit was also found to be preserved for patients with a PS of 2 who accounted for approximately 30% of the enrolled patients.[7] Conversely, with regard to the patients with a PS $\geq$3, there is no evidence as yet to suggest the clinical benefit of administering second-line chemotherapy. In our study, however, response rates of 64% in patients with a PS of 3 (n = 14) and 25% in patients with a PS of 4 (n = 4) were observed. These results suggest that second-line chemotherapy might be beneficial for adequately selected patients with a PS of $\geq$2, although the survival benefit is limited as compared with that for the patients with a PS of 0 to 1. Further studies are required for precise selection of criteria for second-line chemotherapy.

In this study, the survival of patients who received second-line chemotherapy with a PS of 2 to 4 or refractory recurrences was still significantly better than that of those who did not receive second-line chemotherapy. However it was not surprising, because the patient selection for second-line chemotherapy was performed pragmatically, and patients who were thought to be unfit for chemotherapy were not administered second-line chemotherapy. The finding that the nonchemotherapy group had more patients with a PS of 2 to 4 and refractory recurrence, the 2 independent prognostic factors identified in this study, suggests that our patient selection was reasonable.

The prognosis of recurrent SCLC patients is generally poor, and to our knowledge no standard treatment has been established for these patients. In addition to the randomized trial comparing oral topotecan with BSC mentioned above, 2 phase 3 trials for recurrent SCLC have been reported to date.[14,15] A trial comparing intravenous topotecan with the combination of cyclophosphamide, doxorubicin, and vincristine demonstrated comparable response rates and survival; however, intravenous topotecan yielded greater symptomatic improvement for 4 of the 8 symptoms evaluated.[14] In the other trial, comparing oral topotecan with intravenous topotecan, no survival difference was observed.[15] Currently, topotecan is the only drug approved by the US Food and Drug Administration for recurrent SCLC. Recently, however, promising results of phase 2 studies have been reported for drugs other than topotecan for recurrent SCLC. In particular, amrubicin[16,17] and PEI[18,19] have been shown to yield excellent response rates and survival in not only sensitive but also refractory recurrent cases. In Japan, a phase 3 randomized trial comparing topotecan with PEI is now ongoing.

In conclusion, we identified PS and sensitivity to initial chemotherapy as being significant prognostic factors for survival in patients with recurrent SCLC treated with second-line chemotherapy. PS was also found to be predictive in terms of response. In future clinical trials of second-line chemotherapy, both PS and sensitivity to initial chemotherapy should be incorporated as stratification factors. The survival benefit of second-line chemotherapy is limited in patients with impaired PS, even among sensitive recurrence cases. Therefore, careful consideration of the potential risks and benefits is required in the treatment of these patients.

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ANTI TUMOUR TREATMENT

Advances in the treatment of non-small cell lung cancer

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Summary While there have been advances in the treatment of lung cancer, they have been marginal in comparison with recent advances in the chemotherapy and molecularly targeted treatment of breast cancer, colorectal cancer and genitourinary cancer. Lung cancer is an extremely difficult disease to treat, and to obtain positive results and to develop new standard treatment. The results of clinical trial on gefitinib and erlotinib suggest that the evaluation of molecular target drugs seems to be quite difficult in unselected patient population and may be different from cytotoxic drugs. We need to find out specific molecular biomarkers for each drug. With global studies in view, it will be essential to obtain even more significant results by sophisticated clinical trials in selected patient populations and contribute to improving the treatment outcome of lung cancer patients.

It is a well-known fact that lung cancer ranks first as a cause of cancer deaths in developed countries. The number of new cases of lung cancer and the number of deaths from lung cancer are very similar, and the cure rate is regarded to be about 15% even in advanced countries, and 7–8% in developing countries. Despite numerous comparative studies and positive data, very few patients experience any benefit from them. The importance of primary prevention (anti-smoking measures) is recently becoming widely recognized, but an even greater effort is needed. In terms of secondary prevention (lung cancer screening), there are no definitive data in clinical trials, such as quality control, and the conduct of screening examinations has not been reflected in reduced mortality. Under these circumstances the incidence of lung cancer is still rapidly increasing in many countries. A wide variety of clinical trials of treatments have been conducted for lung cancer, which is diagnosed in more than 70,000 new patients annually in Japan. In addition, the results of many of the studies obtained recently have been contrary to expectations, and it seems necessary to reassess the relationship between the pharmacology such as pharmacokinetics, pharmacodynamics and pharmacogenomics and clinical efficacy of each drug in regard to drug therapy.[1]

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