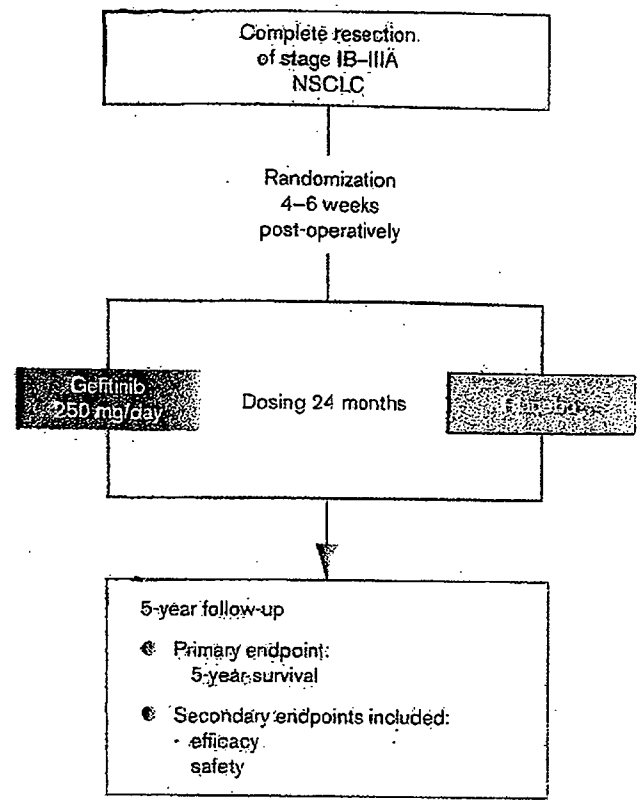
Expanded Access Programme, through which more than 39 000 patients have received gefitinib 250 mg/day on a compassionate-use basis. Furthermore, a retrospective analysis of 9515 US patients who had received gefitinib for 1 year or more via the Expanded Access Programme showed a 1-year survival rate of 33% [12], which compares with the IDEAL studies [10,11]. Recently, Onn *et al.* observed efficacy (16% with objective responses and 45% with stable disease) and a low incidence of grade 3/4 AEs in Japanese patients with NSCLC, most of whom had been treated with second-line gefitinib or above (99% of patients) [13].

To date, there is no experience of using gefitinib in the post-operative adjuvant setting. This phase III trial was initially undertaken to compare survival rates in patients with completely resected stage IB–IIIA NSCLC who had been treated with adjuvant gefitinib 250 mg/day or placebo. However, in October 2002, recruitment was halted following high-profile media activity around reports of gefitinib-related interstitial lung disease (ILD)-type events in patients with advanced or metastatic NSCLC in Japan. In March 2003, the trial was halted because of an increased withdrawal rate. As enrollment could not be resumed until the prospective investigation into gefitinib-related ILD-type events in Japan was completed, the trial was closed. Consequently survival data are not available, although data from patients recruited to the study have been subsequently analyzed for safety.

Fig. 1



Trial design schema.

Methods

Patients

Patients were eligible for inclusion in the trial if they had histologically confirmed NSCLC (post-operative stage IB–IIIA) that had been completely resected 4–6 weeks before the start of treatment. Patients were required to be 20–75 years of age, with a WHO performance status (PS) 0–1, no previous history of chemotherapy, radiotherapy or immunotherapy for NSCLC and no co-malignancies within the past 5 years. All patients gave written, informed consent to participate in the trial, which was conducted in accordance with the Declaration of Helsinki [14] and Good Clinical Practice guidelines.

Study design

This randomized (1:1), double-blind, placebo-controlled, phase III multicenter survival study planned to recruit 670 patients (335 per group) and randomize them to receive either gefitinib (250 mg) or placebo (Fig. 1). Treatment was to be continued for 2 years, or until recurrence/secondary carcinoma or withdrawal criteria were met. An Independent Data Monitoring Committee (IDMC) was set up to assess the efficacy and safety of gefitinib post-operatively, and would advise whether the study should be continued, changed or discontinued.

Assessments

Efficacy

Disease recurrence or secondary carcinogenesis were assessed using X-rays every 3 months during treatment and every 6 months during the follow-up period. Computed tomography (CT) scans were carried out 8 weeks after the first dose (where necessary, the pre-operative thoracoabdominal CT scan could be used), at week 48 during treatment, at week 104 after withdrawal/completion and every 52 weeks thereafter, unless disease recurrence was observed.

Safety

AEs were to be recorded and coded using MedDRA (Medical Dictionary for Regulatory Activities) version 6.0, graded using National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 and assigned causality by the investigators. AEs associated with post-operative complications were defined as events occurring within 90 days after surgery and were recorded without regard to causality. Treatment could be interrupted for up to 14 days, although the IDMC later recommended that drug interruption could be allowed for more than 14 days in cases where ILD-type events were suspected, but could not be confirmed, in order to ensure the safety of

patients who remained in the trial after recruitment was halted. Hematology, biochemistry and urinalysis were also measured at baseline and during the study.

Role of the funding source

This trial was coordinated and supervised by the principal investigators, the IDMC and AstraZeneca personnel, with funding and organizational support from the trial sponsor AstraZeneca.

Results

Patients

Between August and October 2002, 38 patients were randomized into the trial – 18 received gefitinib and 20 received placebo. Patient demography was well balanced between the treatment arms, with the majority of patients having adenocarcinoma histology and WHO PS 1 (Table 1). When the trial was stopped, four patients in the gefitinib arm and 11 patients in the placebo arm were still receiving treatment (Fig. 2). Of the 23 patients who withdrew, 13 did so because of AEs (10 in the gefitinib arm and three in the placebo arm), five were unwilling to continue with treatment (three in the gefitinib arm and two in the placebo arm), two had disease recurrence (both in the placebo arm) and three withdrew for other reasons (one patient in the gefitinib arm had incomplete recovery from surgery that was not drug related, and two patients in the placebo arm had pre-existing interstitial pneumonia and were withdrawn at the request of the sponsor).

Table 1 Patient demography

	Gefitinib 250 mg/day (n=18)	Placebo (n=20)
Sex [n (%)]		
male	14 (77.8)	15 (75.0)
female	4 (22.2)	5 (25.0)
Median age [years (range)]	64.0 (49–73)	62.5 (52–73)
WHO PS [n (%)]		
0	5 (27.8)	9 (45.0)
1	13 (72.2)	11 (55.0)
Histology [n (%)]		
squamous cell carcinoma	4 (22.2)	6 (30.0)
adenocarcinoma	14 (77.8)	14 (70.0)
Stage [n (%)]		
IB	7 (38.9)	8 (40.0)
IIA	2 (11.1)	1 (5.0)
IIB	3 (16.7)	5 (25.0)
IIIA	6 (33.3)	6 (30.0)

Efficacy

From the limited efficacy data, disease recurrence was not seen in patients receiving gefitinib at data cutoff. Three patients who received placebo (one with stage IB and two with stage IIB) experienced disease recurrence – two patients recurred during the trial and one patient recurred after the trial had stopped.

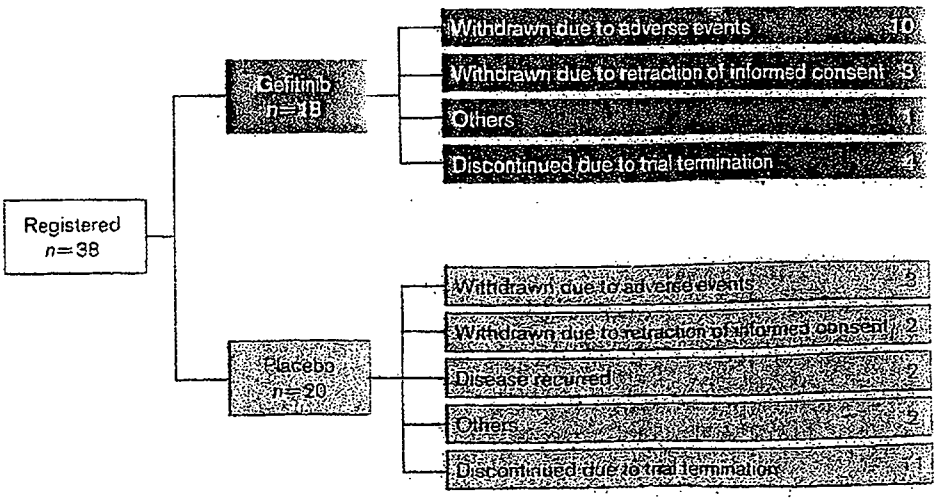
ADRs

No unexpected ADRs were observed and, in general, the frequency of all ADRs was higher for gefitinib versus placebo (Table 2). The most common ADRs were mild to moderate grade 1/2 gastrointestinal and skin disorders. Grade 3/4 ADRs were seen in four patients in the gefitinib arm and one patient in the placebo arm (Table 3), all of whom had treatment withdrawn (the patient with grade 3 eczema had treatment withdrawn due to grade 2 impetigo).

Respiratory ADRs

The majority of respiratory ADRs were grade 1/2 and occurred within 1 month of treatment. In the gefitinib arm, two patients experienced cough (associated with post-operative complications), one patient had dyspnea,

Fig. 2



Trial outcome.

Table 2 Common ADRs occurring in two or more patients

AE (MedDRA term)[a]	Gefitinib 250 mg/day (n=18)	Placebo (n=20)
Abnormal hepatic function	4	0
Acne	2	0
Anorexia	5	1
Cough	2[b]	1
Diarrhea	9	2
Dry skin	3	0
Eczema	8	2
Elevated ALT/AST	2	0
Fatigue	2	0
Gastritis	3[b]	0
Loose stools	4	0
Nausea	3	0
Rash	5	3
Sputum	0	2
Stomatitis	2	0

[a]A patient could have more than one AE.
[b]All were associated with post-operative complications.

Table 3 Grade 3/4 ADRs

AE (MedDRA term)	Grade	Gefitinib 250 mg/day (n=18)	Placebo (n=20)
Abnormal hepatic function	3	1	0
Eczema	3	1	0
Elevated ALT	3	1	0
Neutropenia	3	0	1
Pneumonitis	4	1	0

Table 4 Exposure of patients to gefitinib

	Gefitinib 250 mg/day (n=18)	Placebo (n=20)
Median duration of treatment [days (range)]	86.5 (4–195)	144.0 (20–197)
Dosing period (n)		
<60 days	6	2
60–120 days	9	4
≥ 120 days	3	14
No. dose interruptions (n)		
1	5	6
2	2	2
≥ 3	2	2

Table 5 ADRs leading to patient withdrawals

Adverse event (MedDRA term)	Grade	Gefitinib 250 mg/day (n=18)	Placebo (n=20)
Eczema	2	1	0
Elevated ALT/AST	2	1	0
	3	1	0
Hepatic function abnormalities	2	1	0
	3	1	0
ILD	1	0	1
Impetigo	2	1	0
Neutropenia	3	0	1
Paronychia	2	1	0
Pneumonitis	4	1	0
Pulmonary fibrosis	1	0	1

and one patient experienced grade 4 ILD-type events (pneumonitis) 107 days after starting gefitinib and was withdrawn from the study. The patient with pneumonitis had taken concomitant shosaikoto, a Chinese herbal medicine, and loxoprofen, both of which have previously been shown to induce pneumonitis [15,16]. Twenty-one days later bacterial pneumonia related to methylprednisolone therapy was diagnosed, and the patient subsequently died 37 days later due to both pneumonitis and bacterial pneumonia. In the placebo arm, one patient who experienced cough and grade 1 pulmonary fibrosis had had interstitial changes on their chest X-ray at enrollment, and in a second patient, pre-existing non-specific interstitial pneumonia was exacerbated resulting in grade 1 ILD. In both patients, these conditions persisted following withdrawal of study drug.

Interruptions and withdrawals due to ADRs

ADRs requiring interruptions in therapy were similar between patients receiving gefitinib or placebo (Table 4) and were usually for less than 14 days, although four patients in the gefitinib arm required treatment to be interrupted for 14 days (including one patient whose treatment was interrupted for 20 days). The majority of ADRs leading to withdrawal were usually mild-to-moderate grade 1/2 in severity (Table 5). Grade 3 ADRs leading to withdrawal occurred in two patients receiving gefitinib (hepatic function abnormalities, elevated ALT) and in one patient receiving placebo (neutropenia), and grade 4 pneumonitis led to the withdrawal of one patient who was receiving gefitinib. Following withdrawal of gefitinib treatment, grade 3 abnormal hepatic function and elevated ALT resolved, and grade 3 neutropenia persisted.

AEs associated with post-operative complications

As there are no safety data regarding the use of gefitinib in the post-operative setting, AEs associated with the healing process were examined to provide preliminary safety data on the start of the dosing timing in the adjuvant setting for gefitinib. AEs related to post-operative complications were observed in six patients in the gefitinib arm and four patients in the placebo arm. In the gefitinib arm, the most frequent AEs were grade 1/2 cough (four patients) and gastritis (three patients), and in the placebo arm grade 1/2 pain (three patients). Grade 1 cough, grade 1 supraventricular arrhythmia and grade 2 dyspnea were also experienced by three out of four patients receiving placebo.

Discussion

This trial was designed to compare survival rates in patients with completely resected stage IB–IIIA NSCLC who had received adjuvant therapy with gefitinib 250 mg/day or placebo. However, incidences of ADRs of ILD-

type events in the advanced disease setting have been increasingly reported since gefitinib was launched in Japan, and new recruitment was put on hold on 23 October 2002 at the request of the Ministry of Health, Labor and Welfare. In order to evaluate the ILD and ensure the safety of the trial patients, two separate Co-ordination Committee and IDMC meetings (December 2002 and January 2003) were conducted to discuss the feasibility of continuing the study and management of the trial patients. Based on the updated information on ADRs of interstitial pneumonia, the committees concluded that the study could be continued because the possibility of risk did not exceed that of benefit to enrolled patients. The IDMC also suggested that top priority should be given to assure the safety of the patients receiving gefitinib, and that discontinuation should be considered if flu-like symptoms including difficulty in breathing, fever and coughing occurred.

A 'Supplemental Explanation Sheet and Informed Consent Form' was provided four times to enrolled patients, offered updated information and methods to assure and manage any safety issues, and confirmed the patients' willingness to continue participating in the study. In December 2002, AstraZeneca KK gave the principal investigators the option to suspend gefitinib treatment at once. With the extensive monitoring of the trial patients in terms of safety, there were still an increasing number of withdrawals. In addition, enrollment could not be resumed until the prospective investigation on gefitinib-related ILD was completed. Based on these facts, the sponsor finally decided to terminate the trial in March 2003.

The types of AEs reported in this trial were similar to that already reported in the large phase II IDEAL 1 and 2 trials for patients with locally advanced or metastatic NSCLC [10,11]. Three patients experienced ILD-type events – two in the placebo arm and one patient in the gefitinib arm (this patient was also taking two other medications known to induce ILD) [15,17]. It has generally been observed that a higher frequency of ILD-type events are reported in Japanese patients taking gefitinib compared with those in other south-east Asian countries and the rest of the world (1.6, 0.3, and 0.3%, respectively) [18]. The occurrence of ILD in Japanese patients and the reasons for such an ethnic stratification in ILD incidence following gefitinib treatment require further clarification.

The most common reason for withdrawal in both treatment arms was due to toxicity, with the majority of drug-related AEs being grade 1/2 in severity. In the advanced or metastatic disease setting, few patients who experience grade 1/2 drug-related AEs withdraw from treatment with gefitinib, and in IDEAL 1, which recruited Japanese patients, two out of 103 patients who received gefitinib 250 mg/day withdrew from therapy due to ADRs [18]. Several factors may explain the high number of withdrawals (including withdrawal of treatment for less severe ADRs) reported in this trial data compared with previously reported studies. These reasons include the fact that patients with early-stage NSCLC may be less tolerant of AEs compared with patients with advanced NSCLC who have received prior chemotherapy. In contrast to the other studies, the impact of heavy media coverage surrounding gefitinib-related ILD cannot be ignored.

It has been suggested that the dosage and schedule of gefitinib used in this study may not best suit patients with completely resected NSCLC in terms of tolerability and a number of adjustments may need to be taken into consideration when planning an adjuvant study of gefitinib in the future. It is unlikely that the time frame of 4–6 weeks is too short before starting adjuvant treatment, as other adjuvant trials conducted in Japanese patients have used similar time frames [3,4]. It may be possible to lengthen the duration by which gefitinib could be interrupted for toxicity, since 14 days may be too short for patients recovering from AEs such as hepatic enzyme elevation, or to reduce the dose following toxicity to perhaps 250 mg every other day, although this would require further study into the efficacy of such an approach.

With no experience of using gefitinib in post-operative patients there was a concern that EGFR-TKIs might impact on surgery-related complications (especially on the healing process) due to their mode of action. In order to assess this, the trial was designed to allow a safety review of the first 60 patients. Due to the early termination of the study, we have only 38 patients' (18 on gefitinib) data for review; however, there does not seem to be any impact on surgery-related complications when gefitinib was administered within 4–6 weeks after surgery, as evidenced by a similar number of these AEs that occurred in both groups. This indicates that it may be feasible to administer gefitinib in the adjuvant setting within this time frame.

In conclusion, this is the first study to investigate the use of EGFR-TKIs as adjuvant therapy. Despite the absence of survival data, there were no unexpected AEs seen in the adjuvant setting compared with those already reported for patients with locally advanced or metastatic NSCLC. However, it was observed that there were more AEs leading to withdrawal in the gefitinib arm, even though the majority of AEs were grade 1/2 in severity, suggesting that a daily dose of gefitinib 250 mg may not best suit patients with completely resected NSCLC in terms of tolerability.

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Genomewide cDNA Microarray Screening of Genes Related to Benefits and Toxicities of Platinum-Based Chemotherapy in Patients With Advanced Lung Cancer

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Abstract: The authors conducted a study using cDNA microarray analysis to determine whether expression levels of genes in tumors were correlated with the outcome of chemotherapy. Forty-seven patients were studied, and all except 3 received platinum-based chemotherapy. The expression levels of 1176 genes in transbronchial biopsy specimens of tumors that were obtained before chemotherapy were analyzed using the Atlas Human Cancer 1.2 Array. Multivariate regression analysis revealed that 3 genes were each independent factors related to tumor resistance to chemotherapy and patient survival ($P < 0.01$). Among various chemotherapy-related toxicities, 1, 3, 3, 1, and 1 genes were also revealed to be independent factors that were correlated with neutropenia, anemia, diarrhea, infection, and increased serum creatinine respectively ($P < 0.01$). It is concluded that not only the benefits but also the toxicities of chemotherapy can be predicted by cDNA microarray using tumor specimens obtained before chemotherapy.

Key Words: microarray, gene, lung, cancer

(*Am J Clin Oncol* 2005;28: 367–370)

Lung cancer is a disseminated disease, and most affected patients are candidates for chemotherapy. Although responders to chemotherapy may have a better prognosis than nonresponders,[1] even the most effective chemotherapy cannot always reduce the tumor volume of lung cancer. The properties of cancer cells are determined by complicated interactions among all the gene products they express, and it is certain that many proteins—including enzymes involved in apoptosis, DNA repair, and the metabolism and detoxification of drugs—have individual responses. The cDNA microarray method is now widely used to analyze the expression of thousands of genes simultaneously in cancer tissues, and its development has facilitated the analysis of genomewide expression profiles. Using the cDNA microarray technique on tumor tissues obtained before chemotherapy, we previously identified 3 independent genes, each of which is correlated with chemoresistance and patient survival.[2,3] However, another important aspect of chemotherapy apart from tumor susceptibility and patient survival is the extent of adverse effects. Some cancer patients suffer severe adverse effects of chemotherapy regardless of whether their tumors are chemosensitive. Such patients are unable to receive repeat courses of chemotherapy, even if they have shown a tumor response. Accordingly, it is important to be able to predict not only patients who are likely to respond to chemotherapy, but also those who will probably experience severe treatment-related toxicities.

The current study analyzed the correlation between the expressions of various genes in tumor specimens and chemotherapy-related toxicities, and compared the genes related with the beneficial and toxic effects of chemotherapy.

PATIENTS AND METHODS

Patients

This study was approved by the institutional review board of Kanagawa Cancer Center. Patients with histologically proved lung cancer treated with chemotherapy were entered into the study. All were eligible for treatment. They had an expected survival of at least 6 weeks, measurable lesions, Eastern Cooperative Oncology Group performance status score ≤3 points, a white blood count of ≥4000 cells/μL, hemoglobin ≥10 g/dL, platelet count ≥100,000 platelets/μL, total serum bilirubin less than 2 mg/dL, aspar-

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tate aminotransferase and alanine aminotransferase less than twice the upper limit of the normal range, serum creatinine ≤1.5 mg/dL, and creatinine clearance more than 50 mL/minute. None of the patients had received prior chemotherapy for the primary lesion. Written informed consent for chemotherapy and a genetic analysis of tumor tissue was obtained in every case.

Chemotherapy

All patients with nonprogressive cancer were treated with 2 or more courses of chemotherapy. Response criteria were evaluated according to the World Health Organization criteria.[4] Toxicities were evaluated according to the NCI-CTC version 2 criteria.[5]

Tumor Samples

Transbronchial biopsy specimens of tumors were obtained before chemotherapy. Half the specimens were fixed in formalin for pathologic diagnosis and the other half were immediately frozen for storage at −80°C until genetic analysis.

Extraction and Purification of RNA and Preparation of Probes

The total RNA of each sample was isolated and treated with DNase I to avoid contamination of genomic DNA by silica membrane affinity chromatography using Macherey–Nagel's total RNA isolation kit (MACHEREY–NAGEL GmbH & Co. KG, Germany). One hundred nanograms of the total RNA for each sample was reverse transcribed into cDNA and amplified by SMART polymerase chain reaction (PCR) technology[6] using the Super SMART PCR cDNA Synthesis kit (BD Biosciences Clontech, CA) according to the manufacturer's instructions. Each cDNA sample was subjected to microarray expression profiling using the BD Atlas Human Cancer 1.2 Array (Clontech) based on the manufacturer's protocol described previously.[2,3]

cDNA Microarray

Each labeled probe was then hybridized into a separate Atlas Array. The signal intensity for each spot, which corresponds to each gene examined, was determined using a STORM image analyzer (Amersham Bioscience, Picataway, NJ). The hybridization pattern and signal intensity were analyzed to determine changes in gene expression levels using AtlasImage 2.01 software (CLONTECH, Laboratory, Inc., Japan).

Statistical Methods

t-tests were used to identify differences in mean expression levels among benefits and toxicities of chemotherapy. We compared the differences of gene expression between grade 3 or grade 4 (worst grade) and others for hematologic toxicities, and between grade 0 and others for nonhematologic toxicities. To determine whether gene expression profiles were associated with variety in cases of survival, Kaplan–Meier survival plots and log-rank tests were used. The influence of each gene expression on each outcome of chemotherapy was examined in stepwise multivariate regression analysis. $P < 0.01$ was considered significant.

RESULTS

Between September 2000 and December 2001, 47 patients were registered in the study (Table 1). Thirty-six patients were men and 11 were women, with a median age of 66 years (range, 35–81 years). Eighteen patients had small cell lung cancer (SCLC), and the rest had nonsmall cell lung cancer (NSCLC). Of the patients with SCLC, 2 had limited disease and the other 16 had extensive disease. Of the patients with NSCLC, 12 had locally advanced disease and 17 had metastatic disease. No patients had received prior chemotherapy. All the patients, except for 3 who had been prescribed paclitaxel and irinotecan, were given full-dose platinum-based chemotherapy. Sixteen of the 18 patients with SCLC (89%) and 12 of the 29 patients with NSCLC (41%) responded to chemotherapy.

The expression levels of 1176 genes in the tumor specimens were analyzed using cDNA microarray screening. Four housekeeping genes that were expressed in all 47 tumor

TABLE 1. Patient Characteristics

	No. of Patients
Total	47
Gender	
Male	36
Female	11
Smoker	38
PS(ECOG)	
0	5
1	30
2	9
3	3
Pathology	
SCLC	
Stage	
LD	2
ED	16
NSCLC	
Stage	
IIB/IIIA	4
IIIB	8
IV	17

PS, performance status; ECOG, Eastern Cooperative Oncology Group; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; LD, limited disease; ED, extensive disease.

samples were used as controls for gene expression: ubiquitin, liver glyceraldehyde 3-phosphate dehydrogenase, 23-kDa highly basic protein, 60S ribosomal protein L13A, and 40S ribosomal protein S9.

When we analyzed the relationship between gene expression and chemotherapy-related hematologic toxicity, 2 and 22 genes were identified as showing significantly higher expression in patients with grade 4 neutropenia and grade 3 anemia in comparison with grade 0 to grade 3 neutropenia and grade 0 to grade 2 anemia respectively. We also identified 17, 19, 4, and 1 genes that showed significantly higher expression in patients who experienced diarrhea, infection, increased serum creatinine, and pneumonitis respectively than in patients who did not (grade 0). Stepwise multivariate regression analysis revealed that 1, 3, 3, 1, and 1 genes were independent factors, each of which was correlated with toxicities such as neutropenia, anemia, diarrhea, infection, and increased serum creatinine respectively (Table 2, $P < 0.01$). We were unable to identify any genes that were correlated with thrombocytopenia, emesis, increased total bilirubin, and increased GPT.

As previously presented, stepwise multivariate regression analysis revealed that 3 genes—allograft inflammatory factor 1, HLA-DR antigen-associated invariant subunit, and MHC class HLA-DR-β precursor—were factors independently associated with chemoresistance ($P < 0.0001$, Table 3). When we analyzed the relationship between gene expression level and survival, G1/S-specific cyclin, type II cGMP-dependent protein kinase, and hepatocyte growth factor-like protein were significantly correlated (log-rank test, $P < 0.01$, Table 3). Thus, not only chemotherapeutic benefits but also some toxicities were predicted by cDNA microarray using tumor specimens obtained before chemotherapy.

DISCUSSION

We examined the expression of cancer-related genes in samples of lung cancer obtained before chemotherapy using cDNA microarray screening, and analyzed the relationship between gene expression levels and clinical outcome after chemotherapy. We previously reported 3 genes with expression levels that were each correlated with the tumor response to chemotherapy[2] or patient survival.[3] One surprising finding was that chemoresistant genes related to host immunity were different from survival-related genes. This is because patient survival is influenced by not only the effect of chemotherapy on the tumor but also by tumor growth and metastasis.

The current study revealed some specific genes with expression levels that were correlated with chemotherapy-related toxicity. Cytohesin-1 was identified as a genetic factor that predicted neutropenia resulting from chemotherapy. This is a guanine nucleotide exchange factor that regulates members of the ADP–ribosylation factor family of small GTPases. An analysis of granulocytic maturation of HL-60 cells has revealed a marked increase in the level of cytohesin-1 expression during dibutyryl–cyclic AMP-induced granulocyte differentiation.[7] These data suggest that cytohesin-1 may be useful as a potential marker of granulocytic differentiation.

Three genes—MAD3, DNAX activation protein 12, and interleukin-1β precursor—were identified as predictors of anemia induced by chemotherapy. MAD3 is one of the

TABLE 2. Genes Closely Associated With Chemotherapeutic Toxicities

Factor	Description	Gene Expression (mean ± SD)		Coefficient	SE	*P*
Neutrophil		grade 0–3 (n = 35)	grade 4 (n = 12)			0.0056
	Cytohesin-1	1.8 ± 3.5	6.6 ± 7.9	0.033	0.011	
Hemoglobin		grade 0–2 (n = 43)	grade 3 (n = 4)			<0.0001
	Major histocompatibility complex enhancer-binding protein MAD3	7.0 ± 9.4	43.5 ± 83.7	−0.009	0.004	
	DNAX activation protein 12	10.2 ± 13.1	53.8 ± 63.7	0.005	0.002	
	Interleukin-1 beta precursor	13.1 ± 13.5	529.3 ± 1034.6	0.001	0.0003	
Infection		grade 0 (n = 43)	grade 1–3 (n = 4)			0.0003
	Hemoglobin alpha subunit	7.7 ± 8.8	44.3 ± 61.0	0.007	0.002	
Creatinine		grade 0 (n = 41)	grade 1–2 (n = 6)			0.0021
	Matrix metalloproteinase 10	12.3 ± 19.1	62.2 ± 64.3	0.005	0.001	
Diarrhea		grade 0 (n = 35)	grade 1–3 (n = 12)			0.0002
	ICH-2 protease	16.1 ± 17.5	42.4 ± 36.8	0.008	0.073	
	Interferon-inducible RNA-dependent protein kinase	4.3 ± 6.8	12.9 ± 14.0	−0.028	0.013	
	Collagen 16 alpha 1 subunit precursor	2.8 ± 4.5	15.9 ± 20.0	0.031	0.01	

TABLE 3. Genes Closely Associated With Chemotherapeutic Benefits

Factor	Description	Coefficient	SE	P
Survival	G1/S-specific cyclin D2			0.0055
	Type II cGMP-dependent protein kinase			0.0016
	Hepatocyte growth factor-like protein			0.0075
Tumor effect on chemotherapy	Allograft inflammatory factor 1			<0.0001
	HLA-DR antigen-associated invariant subunit	−0.014	0.002	
	MHC class II HLA-DR-beta precursor	−0.001	0.0003	
		−0.01	0.002	

metaphase checkpoint proteins involved in cell division, and interleukin-1 is one of the monokines that can elicit many of the defective host responses to infection. DNAX activation protein 12 is a membrane adaptor molecule that contains an immunoreceptor tyrosine-based activation motif, which activates calcium signaling in immune cells. However, the mechanisms by which these 3 genes influence the incidence of chemotherapy-related anemia remain unclear.

ICH-2, found to be a predictor of diarrhea, is a novel human gene encoding a member of the interleukin-1β converting enzyme cysteine protease family. ICH-2 mRNA is widely expressed in human tissue and appears to play a primary role in apoptosis.[8] Another predictor of diarrhea, protein kinase regulated by RNA, plays an important role in many cellular processes, including virus multiplication and cell growth, differentiation, and apoptosis.[9] It is also still unclear how these genes, including collagen 16, participate in susceptibility to chemotherapy-related diarrhea.

Although this study revealed a number of genes related to the beneficial and toxic effects of chemotherapy, their mechanisms of action remain to be explained. This may be because we used mononuclear cells from peripheral blood of healthy volunteers as a control for gene expression. A major objective of this study was to clarify predictors of not only beneficial but also toxic effects of cancer chemotherapy. The genetic characteristics of various tissues are believed to differ from one another. Therefore cancer cells need to be examined to clarify the factors related to tumor susceptibility to chemotherapy, and blood cells need to be examined for susceptibility to hematologic toxicities. Malignant tumor tissues are heterogeneous and contain a number of cell types, and specimens of lung cancer obtained by transbronchial biopsy are not considered to reflect the general characteristics of tumor tissue. The fact that genetic information on tumor cells can predict not only tumor susceptibility to chemotherapy but also toxicity suggests that certain genetic characteristics may be common to all somatic cells, irrespective of whether they are malignant or normal. If this hypothesis is correct, then nonmalignant normal cells may also be used for analysis of informative genetic factors that can predict the antitumor effects and toxicities of chemotherapy.

We need to undertake prospective evaluations to determine whether the genes revealed in this study are truly important and potentially useful for predicting the beneficial or toxic effects of chemotherapy. Accumulation of such data could eventually allow chemotherapy to become "personalized" using anticancer drugs that would be effective and nontoxic in individual patients.

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Expert Opinion

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Gefitinib in non-small cell lung cancer

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Gefitinib (Iressa™), an orally-active tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR), is the first approved molecular-targeted drug for the management of patients with advanced non-small cell lung cancer (NSCLC). Two Phase II trials (IDEAL [Iressa Dose Evaluation in Advanced Lung Cancer]-1 and -2), evaluated the efficacy of gefitinib in advanced NSCLC patients who received ≤ 2 (IDEAL1) or ≥ 2 (IDEAL2) previous chemotherapy regimens. The response rate and disease control rate in IDEAL1 and -2 was 18/12% and 54/42%, respectively. The median survival time and one-year survival rate in both studies were ~ 7 months and 30%, respectively. As gefitinib has demonstrated antitumour activity and an acceptable tolerability profile not typically associated with cytotoxic adverse events, such as hematological toxicities, combinations with cytotoxic drugs have been evaluated. Disappointingly, in chemotherapy-naive patients with advanced NSCLC, gefitinib 250 and 500 mg/day combined with platinum-based chemotherapy (gemcitabine/cisplatin or paclitaxel/carboplatin) did not produce prolonged survival, compared with chemotherapy alone in two large, randomised, placebo-controlled, multi-centre Phase III trials (INTACT [Iressa NSCLC Trial Assessing Combination Treatment]-1 and -2). Furthermore, in a recent randomised, placebo-controlled, Phase III trial (ISEL: IRESSA Survival Evaluation in Lung cancer), gefitinib failed to prolong survival compared with placebo in patients with advanced NSCLC who had failed one or more lines of chemotherapy. Subgroup analysis of ISEL suggested improved survival in patients of Asian origin and non-smokers. In addition, subset analyses of IDEAL and several retrospective studies have indicated that female gender, adenocarcinoma histology (especially bronchial alveolar carcinoma), non-smoker status and Asian ethnicity are factors which predict to response to gefitinib. Two types of somatic mutation clustered around the ATP binding pocket in the tyrosine kinase domain of the EGFR gene have been reported as possible surrogate biological markers for predicting response to gefitinib. Appropriate patient selection by clinical characteristics or genetical information is needed, both for future clinical trials of gefitinib and its routine use in the clinic among patients with advanced NSCLC.

Keywords: chemotherapy, epidermal growth factor receptor tyrosine kinase inhibitor, gefitinib, molecular targeted drug, non-small cell lung cancer

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1. Introduction

Metastatic lung cancer remains the most common fatal cancer in many countries and ~ 80% are categorised as non-small cell lung cancer (NSCLC). Systemic platinum (cisplatin)-based chemotherapy for patients with advanced NSCLC prolongs survival and palliates symptoms, compared with the best supportive care [1]. Platinum (cisplatin or carboplatin) combinations with the new cytotoxic drugs of the 1990s, such as gemcitabine [2], vinorelbine [3], paclitaxel [4], docetaxel [5] or irinotecan [6], yield

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higher response rates in stage IV NSCLC, and show superior survival benefits compared with cisplatin [7], single use of the new drug [8] or platinum combinations with old drugs [9]. None of these new combinations offer a significant advantage over the others in terms of response rate and overall survival [10,11], suggesting that each of them may serve as a current standard regimen for metastatic NSCLC. Although combination chemotherapy produces prominent toxicities, including severe nausea, vomiting, renal dysfunction, neuropathy or myelosupression, median survival time (MST) rarely exceeds 12 months, with the vast majority of patients dying within 24 months of diagnosis. Thus, there is an urgent need for novel anticancer drugs that have improved activity and low toxicity and that can help to maintain a reasonable quality of life (QOL) during the relatively short lifespan remaining for most patients with advanced metastatic NSCLC.

Gefitinib (Iressa™) is an orally active epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) that has produced good response rates and symptom improvement in patients with relapsed NSCLC in Phase II trials [12,13]. Gefitinib is one of the molecular targeted drugs that theoretically attack only malignant cells, and, therefore, should show only mild toxicity. Gefitinib produces specific adverse effects such as rash, diarrhoea, and symptomatic increases in liver transaminases but most of them are generally mild [12,13]. Erlotinib is also an EGFR-TKI drug, which has a similar chemical structure, mechanism of action and toxicity profile [14]. The outcome of the randomised, placebo-controlled Phase III trials, which evaluated the efficacy of the two EGFR-TKI drugs in relapsed or refractory NSCLC, were different: one was positive [15] (erlotinib; BR21) and the other was negative (gefitinib; ISEL). However, the survival benefit of these two EGFR-TKIs in patients with refractory NSCLC was small and could easily be influenced by difference in the clinical characteristics of the trial populations. The efficacy of molecular-targeted drugs theoretically depends on the status of the target molecule, as seen with trastuzumab (Herceptin™) [16], the monoclonal antibody against HER2; or imatinib (Gleevec™) [17], the Bcr-Abl tyrosine kinase inhibitor. In the case of gefitinib, the total EGFR expression level, as determined by immuno-histochemistry in the tumour tissue, did not correlate with the response to gefitinib in the Phase II IDEAL (Iressa Dose Evaluation in Advanced Lung Cancer) trials [18]. Recent studies showed that specific somatic mutations in the tyrosine kinase domain of the EGFR gene correlate with the response to gefitinib [19,20]. This review concentrates on the past clinical studies of gefitinib and also discusses proposed future uses for the drug.

2. Mechanism of action

The EGFR-family consists of four structurally similar TK proteins including HER1 (EGFR, erbB1), HER (erbB2, Her2/neu), HER3 (erbB3) and HER4 (erbB4) [21]. The specific ligands for EGFR are EGF, TGF-α, HB-EGF, amphiregulin and betacellulin; however, the ligand for HER2 has not been identified. Each HER exists in monomer form at the surface of cellular membranes. When a ligand binds to the extracellular domain, the HER either forms a homodimer or heterodimers with other members of the HER family [22]. Ligand binding causes autophosphorylation of TK in the intra-cytoplasmic domain near the C-terminus of the receptors. Overexpression of EGFR or HER2 has been reported in many solid tumours [23] and is related to poor prognosis [24]. The members of the EGFR family are typical TK receptors, which have an extracellular ligand-binding domain and a cytoplasmic domain (Figure 1). The amino-terminal extracellular domain of EGFR consists of 622 amino acids and two cysteine-rich regions that form the ligand-binding domain. The cysteine-rich domain 1 is deleted in EGFR vIII [25], which is a variant receptor that is often detected in malignant tumours. The transmembrane region is a single α-helix. The cytoplasmic domain contains a kinase region and a carboxy-terminal tail that contains several tyrosine phosphorylation sites. Gefitinib is a small molecule (~ 447 Da) that inhibits the intracellular TK domain of EGFR; it is orally-active and was the first HER-targeted TKI to receive regulatory approval [26]. This small molecule is a synthetic anilinoquinazoline (Figure 2) that inhibits the

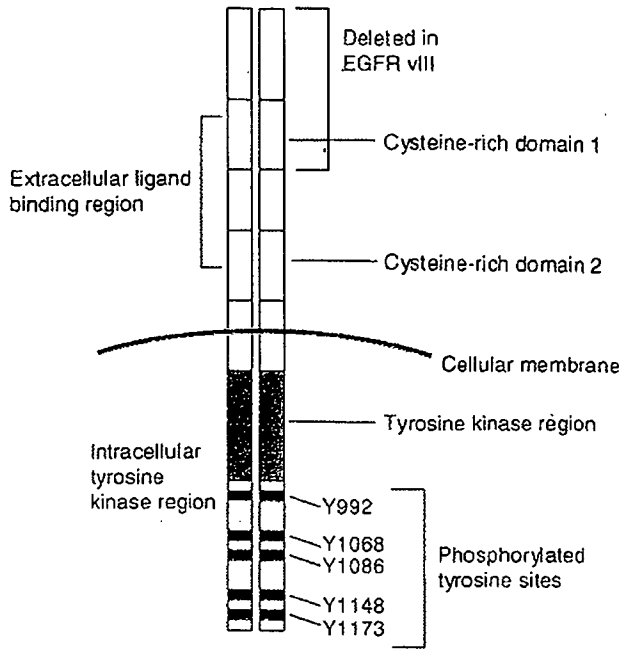

Figure 1. HER1 has an extracellular ligand-binding domain and a cytoplasmic domain. The extracellular domain consists of two cysteine-rich regions, although the amino-terminal domain of the two (domain 1) is deleted in EGFR vIII. The transmembrane region is a single α-helix. The cytoplasmic domain contains a kinase region and a carboxy-terminal tail, which contains several tyrosine phosphorylation sites.

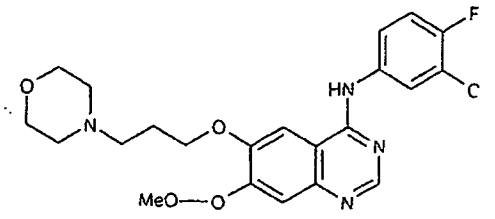

Figure 2. Chemical structure of gefitinib.

Table 1. Kinase inhibition by gefitinib *in vitro*.

Enzyme	IC_{50} (μM)
EGFR	0.023
HER2 (erbB2)	1.2 – 3.7
VEGFR	
KDR	3.7 – 33
c-fit	> 100
PKC	> 100
Raf	> 100
MEK	> 100
Erk2	> 100

isolated EGFR tyrosine kinase with a 50% inhibitory concentration (IC_{50}) of 23nM (Table 1) [27].

3. Pharmacokinetic Properties

Gefitinib was absorbed moderately slowly with a median t_{max} of 4 h (range 5 – 12 h) after a single dose of 50 mg/day [28]. The C_{max} of gefitinib was 31 ng/ml after a single 50 mg dose, and increased dose-proportionally in patients with solid tumours receiving 50, 100 and 225 mg/day (C_{max}: 31, 43 and 150 ng/ml, respectively). Repeat administration of gefitinib (50 – 700 mg/day) for 14 days resulted in dose-related increases in the mean C_{max} (from 60 to 1156 ng/ml), which are at least a two-fold increase compared with single dosing. Although the C_{max} of gefitinib was more than doubled after repeat administration, compared with single-dose administration, t_{max} and $t_{1/2}$ changed only slightly. The t_{max} and $t_{1/2}$ after repeat administration (50 – 700 mg/day) were 6 h (range 5 – 7 h), and 50.1 h (range 27.8 – 79.7 h), respectively. Steady-state plasma concentrations were achieved in 7 – 10 days. The area under the plasma concentration-time curve (AUC) from 0 to 24 h with repeat administration (50 – 700 mg/day) increased dose-proportionally (from 1021 to 21,580 ng/h/ml), with up to six-fold interpatient variability at each dose level. Pharmacokinetic parameters developed in Japan [28] were comparable with results from parallel studies in the US [26] and Europe [29]. Gefitinib has a mean volume of distribution at steady-state of 1400 l, indicating extensive distribution into tissues. Plasma protein binding is ~ 90%. With regard to tumour penetration by the drug, concentrations of gefitinib were up to 12 times greater in tumour tissues than in the plasma of a mouse NSCLC xenograft model [30].

Hepatic metabolism, predominantly by the cytocrome P450 (CYP) isozyme CYP3A4, is the main route of clearance of gefitinib. O-Desmethyl gefitinib, the major metabolite identified in human plasma, is 14-fold less potent than gefitinib in inhibiting EGFR-stimulated cell growth; hence, it is unlikely to contribute significantly to the pharmacological activity of the parent drug. Dosage adjustment is not required on the basis of patient age, bodyweight, gender, ethnicity, renal function or moderate-to-severe hepatic impairment due to liver metastases.

Ranson and Wardell [31] have reviewed the pharmacokinetic interactions between gefitinib and other agents. The AUC of gefitinib decreased by 85% when the drug was co-administered with rifampicin (a potent CYP3A4 inducer) but increased by 78% when gefitinib was co-administered with itraconazole (a potent CYP3A4 inhibitor) in healthy volunteers. A dosage increase from 250 to 500 mg/day might, therefore, be considered when administering potent CYP3A4 inducers with gefitinib in the absence of severe adverse drug reactions. However, caution should be used when administering gefitinib with potent CYP3A4 inhibitors. Elevated international normalised ratio and/or bleeding events have been reported in some patients concurrently receiving gefitinib and warfarin.

4. Pharmacodynamic properties

In preclinical studies [32], gefitinib dose-dependently inhibited cellular proliferation and tumour growth, increased apoptosis and showed a synergistic effect with cytotoxic drugs or irradiation in human NSCLC cell lines and xenografts. Some reports suggested that gefitinib has growth-inhibitory activity against cisplatin- and docetaxel-resistant PC-9 or PC-14 cell lines and antitumour activity in PC-9 xenografts. The mechanism of the antitumour activity of gefitinib has not been fully characterised. The response to gefitinib was not correlated with the level of EGFR expression in xenograft studies [33] or in Phase II clinical studies in advanced NSCLC [18]. Several recent studies [19,20] suggest that the presence of somatic mutations in the TK domain of the EGFR gene predicts tumour responsiveness to gefitinib in most cases; similar results have also been reported for erlotinib, a second orally-active EGFR-TKI [34]. Combined data from the two reports showed that 13 of 14 patients with gefitinib-sensitive NSCLC had EGFR-TK somatic mutations, compared with 0 of 11 patients with no response [19,20].

5. Phase I clinical trials for solid tumours

Phase I trials have evaluated both intermittent and continuous administration [28,29] of gefitinib (14 days of treatment

Table 2. Phase II study of gefitinib (IDEAL1 and IDEAL2).

Treatment history	Dose (mg/day)	IDEAL1	IDEAL2
		1 – 2 regimens (+ platimum based CT)	≥ 2 regimens (+ platinum-based CT, + docetaxel)
Location		Japan/Europe	US
Number of patients		210	216
Response rate (%)	250 500	18.4 19	11.8 8.8
Median survival time (months)	250 500	7.6 7.9	6.1 5.9
Symptom improvement (%)	250 500	40 37	43 35

followed by 14 days observation, once daily for 28 days, respectively). Patients were eligible if they had tumours known to express EGFR, such as NSCLC, breast, colon, ovarian, prostate, and head and neck cancers, but they were not prescreened for detectable EGFR in the tumour before study entry. Both schedules of gefitinib administration were tolerated with minor toxicities such as grade 1 – 2 skin rash, nausea, vomiting and diarrhoea. The most frequent toxicity was skin rash. Most dose-limiting toxicity (DLT) occurred at doses of 700 mg/day with the most common DLT being grade 3 diarrhoea (in a Japanese trial, DLT were both diarrhoea and increase in liver transaminases). Although not a primary end point for these studies, objective responses, as well as disease stabilisation, especially in patients with adenocarcinoma of NSCLC, were seen at several dose levels and were frequently associated with improvement of disease-related symptoms.

6. Phase II clinical trials for second- or third-line advanced non-small cell lung cancer

Two large Phase II trials have examined gefitinib (250 and 500 mg/day) monotherapy in patients with NSCLC who were previously treated with one or two chemotherapy regimens (at least one containing platinum) in Europe, Japan, Australia and New Zealand (IDEAL1 [12]), or who had been treated with two or more previous regimens containing platinum and docetaxel in the USA (IDEAL2 [13]). The overall response rates with gefitinib 250 and 500 mg/day were 18.4 and 19.0%, respectively, in IDEAL1 and 11.8 and 8.8%, respectively, in IDEAL2 (Table 2). At 250 mg/day, the median survival time (MST) was 7.6 months in IDEAL1 and 6.1 months in IDEAL2. The MST achieved with gefitinib in IDEAL1 was similar to that reported for docetaxel, which is a key drug in the second line chemotherapy in patients with NSCLC. The most frequently reported adverse events in the IDEAL trials were skin rash, diarrhoea, increased transaminases and nausea. Most of the toxicities were mild (grade 1/2). However, more grade 3/4 events occurred in patients who received gefitinib 500 mg/day than in those receiving 250 mg/day.

Gefitinib demonstrated significant improvement in disease-related symptoms and quality of life in IDEAL1 and IDEAL2, assessed by the Lung Cancer Subscale (LCS), or the Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire. In both IDEAL trials, 40.3 and 43.1% of patients reported symptom improvement by LCS, respectively, and 23.9 and 34.3% of patients reported improved quality of life by FACT-L, respectively. Improvement in disease-related symptoms and quality of life was associated with an objective tumour response and stable disease. Symptom improvement also correlated with longer progression-free survival.

7. Phase III clinical trials for first-line advanced non-small cell lung cancer

In preclinical studies, it was noted that gefitinib enhanced the cytotoxic activity of different chemotherapeutic drugs such as platinum compounds, gemcitabine and taxanes. This synergy with chemotherapy was independent of the level of EGFR expression. These findings provided a strong rationale for combining gefitinib with standard chemotherapy regimens as first-line therapy in advanced NSCLC. The INTACT [35,36] studies investigated whether concurrent use of gefitinib and standard chemotherapy in first-line chemotherapy could improve the outcome of patients with advanced NSCLC. In the INTACT1 [35] randomised Phase III study, cisplatin/gemcitabine + gefitinib were administered, while the INTACT2 [36] study administered carboplatin/paclitaxel and gefitinib. In both INTACT1 and -2 studies, gefitinib failed to prolong survival time and time to progression of the patients with advanced NSCLC by concurrent use of platinum-based doublet chemotherapy in first-line chemotherapy.

8. Tolerability

Gefitinib was generally well tolerated in Phase II clinical trials [12,13]. The most frequently reported adverse events in both trials were skin rash, diarrhoea, increased liver transaminases and nausea. Most of the toxicities were mild (grade 1/2). However, more grade 3/4 events occurred in patients who received gefitinib 500 mg/day than in those receiving 250 mg/day. As there was no significant difference in overall survival between the two dosages, a recommendation for future clinical trials or practical use has been set at 250 mg/day. This toxicity typically occurred within the first month of the treatment. Liver function usually recovered after discontinuation of therapy [12]. The tolerability profile of gefitinib 250 or 500 mg/day combined with conventional chemotherapy was similar to that of chemotherapy alone in INTACT1 [35] and -2 [36] trials. No new clinically significant and/or unexpected adverse events from combination with chemotherapy were seen. The frequency of skin rash had no relationship with the response to gefitinib, in contrast to erlotinib [14,34].

Interstitial lung disease (ILD) has been reported in patients receiving gefitinib; however, the incidence was much higher in Japan than in the Western countries. Up to September 2003, the worldwide incidence of ILD among 92,750 patients who received gefitinib in the expanded access programme (EAP) was 0.99%: approximately a third of these cases were fatal [37]. In contrast, in retrospective analyses [38] of 1976 patients in Japan, ILD developed in 69 patients (3.5%), 25 of whom (1.3%) died. Multivariate analysis [38] with a logistic model indicated that risk factors for ILD were male gender, current or past smoking and hypoxia, with odds ratios of 3.17, 4.38 and 1.85, respectively. Multivariate analysis with the Cox model revealed that prognosis was poor in patients who had an Eastern Cooperative Oncology Group (ECOG) performance status of 2 – 4 at the start of gefitinib treatment or had taken gefitinib for < 2 weeks when ILD developed.

9. Phase III clinical trials for relapsed or refractory advanced non-small cell lung cancer

The ISEL (IRESSA Survival Evaluation in Lung cancer) study investigated the survival benefit of gefitinib 250 mg/day as monotherapy in patients with advanced NSCLC who had failed one or two lines of chemotherapy. The proportions of second- and third-line patients were ~ 50/50. About 1700 patients were enrolled; the study population was representative of the general advanced NSCLC population, and patients who enrolled were either intolerant of, or refractory to, their most recent prior chemotherapy regimen. As a result, although there was some improvement in survival with gefitinib, in comparison with placebo, this failed to reach statistical significance in the overall population (HR 0.83, p = 0.11, median 5.6 versus 5.1 months), or in patients with adenocarcinoma (HR 0.83, p = 0.07, median 6.3 versus 5.4 months). A placebo-controlled, randomised Phase III clinical trial [15] of erlotinib had recently reported a survival benefit of erlotinib in patients with relapsed NSCLC (BR21). Prospective subgroup analyses of the ISEL trial suggested survival benefit of gefitinib in patients of Oriental origin and in patients who had never smoked.

10. Expanded access programme

The compassionate-use global EAP provides data on the effectiveness of gefitinib 250 mg/day monotherapy in patients with advanced NSCLC, treated in everyday clinical practice. Treated individuals included refractory cases from previous chemotherapy, chemotherapy-naive cases, and elderly or poor performance status patients. In the US EAP [39], 21,064 evaluable patients were registered, 52.4% were men, 87.8% were white, 23.1% were aged > 74 years and 71.8% had stage IV disease. The median survival time was 5.3 months and 1-year survival rate was 29.9%. Subgroup analysis of the survival data from patients recruited into the EAP at least 1 year prior to analysis found that the median survival duration was longer in women than in men (7.6 versus 4.8 months), and longer in stage IIIa and IIIb than in stage IV patients (9.2 and 7.4 versus 5.1 months), (p < 0.0001). The 1-year survival rates in White, Black, Hispanic, Asian and Oriental patients was 31.4, 30.9, 35.2, 56.7 and 52.2%, respectively.

11. Gefitinib in elderly or poor performance status patients

In the large-scale US EAP [39], gefitinib monotherapy has shown promising efficacy in some subsets of patients with advanced NSCLC, namely elderly or poor performance status (PS) patients [40] in a chemonaive setting. One- and two-year survival of patients who were PS2 (n = 222 /21,064) was 17.6 and 5.5%, respectively. A small retrospective study in Japan [41] showed that the response rate of gefitinib was similar between young and elderly patients. Several reports have shown a utility of gefitinib in elderly patients [42-44]. For elderly patients (> 70 years old), a randomised, Phase II study comparing gefitinib with vinorelbine (INVITE) in patients with NSCLC is ongoing (Table 3). Another randomised Phase II trial is examining gefitinib versus placebo (INSTEP) as the first-line chemotherapy for NSCLC patients with PS2 or 3.

12. Predictive factors of response to gefitinib

Multivariate analysis of IDEAL1 shows that female gender or adenocarcinoma histology are significant predictive factors of response to gefitinib. Further studies [41] also showed that Asian ethnicity, good performance status, low smoking history, and bronchial alveolar carcinoma [45] also are clinical predictive factors. Genetically predictive factors of response to gefitinib have been the focus of two recent papers [19,20]. Tumour specimens from 25 NSCLC patients prior to treatment with

Table 3. Major ongoing or planned trials of gefitinib in patients with non-small cell lung cancer.

Study number	Phase	Number of patients	Regimens	Primary end point
Adjuvant therapy: stage I – IIIA completely resected				
8R19 (NCIC, EORTC)	III	1160	GEF versus PL	OS
First-line: stage IV				
0711	RII	200	GEF + BSC versus PL + BSC	PFS
INVITE (aged > 70 years)	RII	192	GEF versus VNR	PFS
INSTEP (PS 2 or 3)	RII	200	GEF versus PL	PFS
N Natale's group	RII	250	GEF versus CBDCA + GEM	PFR
V Hirsh's group	RII	170	GEF versus CBDCA + TXL	DCR
WJTOG 0403 (selected by mutation)	II	25/120	GEF	OR
Second- or third-line				
INTEREST	III	1440	GEF versus DOC	OS
Maintenance: stage III				
SWOG0023	III	840	GEF versus PL, after treatments with CE + TRT → DOC	OS
Sequence or maintenance: stage IV				
See Table 4				

BSC: Best supportive care; CBDCA: Carboplatin; CE: Cisplatin + etoposide; DCR: Disease-free survival; DOC: Docetaxel; GEF: Gefitinib; GEM: Gemcitabine; OR: Objective response; OS: Overall survival; PFS: Progression-free survival; PL: Placebo; RII: Randomised Phase II; SWOG: Southwest Oncology Group; TRT: Thoracic radiation therapy; TXL: Paclitaxel; VNR: Vinorelbine; WJTOG: West Japan Thoracic Oncology Group.

gefitinib (16 and 9 patients from the respective studies of Lynch *et al.* [20] and Paez *et al.* [19], of whom 9 and 5, respectively, responded to gefitinib) were analysed for the presence of somatic mutations at the kinase domain (exons 18 – 24) in the EGFR gene. The somatic mutations were identified in the TK domain of the EGFR gene in 13 of 14 patients who had major response to gefitinib, compared with 0 of the 11 patients who did not show a major response ($p < 0.001$). Mutations were either in-frame deletions in exon 19, or amino acid substitutions in exons 18 and 20 around the ATP-binding pocket of the TK domain. Similar mutations were detected in tumours from 2 of 25 North American patients in the study [20]. Paez's group [19] also reported similar somatic mutations in the EGFR gene in 15 of 58 unselected tumours from Japan and 1 of 61 from North American patients. The mutations appeared more common in women (20 versus 9%), patients with adenocarcinoma (21 versus 2%), and non-smokers (54 versus 11%). These mutations in the EGFR TK domain were not identified in 95 primary tumours and in 108 cancer cell lines (breast, colon, pancreas, kidney, prostate, head and neck and brain). Substitution mutations changing leucine 858 to arginine (L858R), guanine 719 to serine (G719S), and leucine 861 to glutamine (L861Q) were in the activation and glycine-rich P loops, which are important for auto-regulation. In-frame deletions were clustered in the region spanning codons 746 – 750 (ELREA) around the active site of kinase, which is conserved among vertebrates. Transfection of dominant negative EGFR mutants into Cos-7 cells have enhanced tyrosine kinase activity (increased the phosphorylated population of EGFR) and increased sensitivity to gefitinib. Tumour samples were available from 228 patients in the randomised Phase III trial (TRIBUTE), which evaluated the efficacy of erlotinib combined with carboplatin (CBDCA) and paclitaxel (TXL) in the first-line treatment of advanced NSCLC. EGFR mutations in exons 18, 19 and 21 of the TK domain were detected in 15/114 patients treated with CBDCA + TXL + erlotinib, and in 14/113 patients treated with CBDCA + TXL only. Both the 15 and 14 patients experienced significant longer survivals compared with the patients who did not have EGFR mutations, suggesting that the alteration of the EGFR gene is not only a predictive response factor to gefitinib, but also a prognostic factor in advanced NSCLC.

Other molecular alterations, including hetero-dimerisation with other members of this receptor family such as HER2 or HER3, amplification of EGFR, increased expression of ligands, expression of other receptor (e.g., IGFR-1), phosporylation of EGFR or downstream pathways of EGFR (e.g., pAKT and MAPK) were also reported as the predictive markers of response to gefitinib. Total EGFR or HER2 expression level or both of them by immunohistochemistry

Table 4. Maintenance or sequence therapy by gefitinib after platinum doublets as first-line chemotherapy.

	EORTC	SWOG	WJTOG
Population	First-line NSCLC	First-line NSCLC	First-line NSCLC
Design	Maintenance	Maintenance	Sequence
Randomisation	Post-fourth course	Post-fourth course	Pre-first course
Phase	III	III	III
Stage	IIIB/IV	IIIB/IV	IIIB/IV
Performance status	0 – 2	0 – 1	0 – 1
Regimens	4 Cycles* → GEF versus BSC	4 Cycles* → GEF versus BSC	3 Cycles + GEF versus 3 – 6 cycles
Number of patients	736	750	600
Primary end point	OS	PFS	OS

*Four cycles of platinum doublets treatment as a standard regimen for chemonaive patients with advanced NSCLC.
BSC: Best supportive care; EORTC: European Organisation for Research and Treatment of Cancer; GEF: Gefitinib; NSCLC; Non-small cell lung cancer; OS: Overall survival; PFS: Progression-free survival; SWOG: Southwest Oncology Group; WJTOG: West Japan Thoracic Oncology Group.

in the tumour tissue did not correlate with the response to gefitinib [46]. Recent preclinical data [47] suggested that gefitinib might be more sensitive in EGFR homodimer or EGFR/HER2 heterodimer than in EGFR/HER3 heterodimer. EGFR amplification was also reported as one candidate of the predictive maker of response to gefitinib; however, a high score of EGFR amplification is not frequent in NSCLC, further research is needed.

The phosphorylation status of two major downstream signalling pathways, namely the phosphatidylinositol 3-kinase/Akt (Akt) and mitogen-activated protein kinase (MAPK) cascades, have also undergone initial investigations as potential molecular markers of tumour response to gefitinib [48]. Among 103 evaluable patients, Akt, but not MAPK, activation predicted increased tumour sensitivity to gefitinib. Consistent with this finding is a recent preclinical report that antiapoptotic (Akt and STAT) but not proliferative (Erk/MAPK) pathways are selectively activated by gefitinib-sensitive EGFR mutations [49].

EGFR polymorphisms in the intron 1 would be associated with an efficacy or skin toxicity of gefitinib in 19 patients with colon cancer [50]. Two recent reports [51,52] have also suggested that acquired resistant to gefitinib correlates a secondary mutation in EGFR kinase domain, resulting in threonine to methionin amino acid change at position 790 (T790M).

13. Future clinical trials

Several ongoing or planned studies aim to identify the optimal use of gefitinib in different clinical settings, including adjuvant, first-line monotherapy, second- or third-line settings or maintenance therapy in patients with locally advanced (stage III) or metastatic (stage IV) NSCLC (Table 3). The efficacy of gefitinib as adjuvant therapy is being evaluated in a large-scale, placebo-controlled, randomised trial (NCIC BR19). A large, randomised, Phase III trial (gefitinib versus docetaxel) in patients with advanced NSCLC who had failed one or more lines of chemotherapy is also ongoing in a Western country (INTEREST: Iressa NSCLC Trial Evaluating Response and Survival against Taxotere) and Japan (V15-32). The feasibilities of toxicity or schedule were reported in a Phase I study of concurrent gefitinib, carboplatin, paclitaxel and radiation [42]. Platinum plus etoposide combined with concurrent chest radiation and then treated with docetaxel, and followed by gefitinib in patients with stage III NSCLC are being evaluated by SWOG, and in trials of platinum doublet followed by gefitinib in patients with stage IV NSCLC (Table 4) are ongoing in EORTC, SWOG and WJTOG (Japan) as randomised Phase III trials.

14. Expert opinion and conclusion

Gefitinib represents a significant advance in the treatment of patients who have failed treatment with both platinum-based and docetaxel chemotherapy; however, in the Phase III ISEL trial, the survival benefit for this population was small, at least in patients in Western countries. On the contrary, erlotinib, which has a similar chemical structure and mechanism of action as gefitinib, has revealed a significant survival benefit. Both Phase III monotherapy trials have not yet been published in full. Additional data are needed about EGFR mutation ratio of the patients in both trials. The recommended dose of gefitinib is much lower than the most tolerable dose of it, in comparison with erlotinib. The dose difference might influence in different outcomes. EGFR somatic mutations might be susceptibility markers to predict response to gefitinib but prospective studies are needed to

confirm their utility and to determine the feasibility of an assay for clinical use. Patient selections according to clinical factors (e.g., female, adenocarcinoma and non-smoker) or genetical factors (e.g., somatic mutation) may be necessary to optimise the use of gefitinib, corresponding to the first step to tailoring treatment to individual patients.

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Original Articles

Importance of the Initial Volume of Parotid Glands in Xerostomia for Patients with Head and Neck Cancers Treated with IMRT

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Objective: Our aim was to evaluate predictors of xerostomia in patients with head and neck cancers treated with intensity-modulated radiation therapy (IMRT).
Methods: Thirty-three patients with pharyngeal cancer were evaluated for xerostomia after having been treated with IMRT. All patients were treated with whole-neck irradiation of 46–50 Gy by IMRT, followed by boost IMRT to the high-risk clinical target volume to a total dose of 56–70 Gy in 28–35 fractions (median, 68 Gy). For boost IMRT, a second computed tomography (CT-2) scan was done in the third to fourth week of IMRT. Xerostomia was scored 3–4 months after the start of IMRT.
Results: The mean doses to the contralateral and ipsilateral parotid glands were 24.0 ± 6.2 and 30.3 ± 6.6 Gy, respectively. Among the 33 patients, xerostomia of grades 0, 1, 2 and 3 was noted in one, 18, 12 and two patients, respectively. Although the mean dose to the parotid glands was not correlated with the grade of xerostomia, the initial volume of the parotid glands was correlated with the grade of xerostomia ($P = 0.04$). Of 17 patients with small parotid glands (≤38.8 ml) on initial CT (CT-1), 11 (65%) showed grade 2 or grade 3 xerostomia, whereas only three (19%) of 16 patients with larger parotid glands showed grade 2 xerostomia ($P < 0.05$). The mean volume of the parotid glands on CT-1 was 43.1 ± 15.2 ml, but decreased significantly to 32.0 ± 11.4 ml (74%) on CT-2 ($P < 0.0001$).
Conclusions: Initial volumes of the parotid glands are significantly correlated with the grade of xerostomia in patients treated with IMRT. The volume of the parotid glands decreased significantly during the course of IMRT.

Key words: IMRT – xerostomia – parotid glands – head and neck cancer

INTRODUCTION

Xerostomia is the most common late toxic effect of radiation therapy (RT) in patients with head and neck cancers. Decreased saliva output affects every aspect of life, including speech, nutrition, taste, sleep, mastication and deglutition (1–5). Xerostomia also affects aspects of patients' health-related quality of life (QOL), such as pain, emotion and communication (4). Thus, QOL after RT is largely related to xerostomia in survivors.

Recent technological advances have led to the successful clinical use of intensity-modulated RT (IMRT), an advanced form of conformal RT. The initial clinical results of IMRT have been encouraging. IMRT reduces late salivary toxicity without compromising tumor control in patients with head and neck cancers (1,6–9).

To evaluate the predictors of xerostomia in patients with head and neck cancers treated with IMRT, we compared dosimetric parameters of the parotid glands with the grade of xerostomia.

PATIENTS AND METHODS

Since December 2000, 33 patients with head and neck cancers who were treated with whole-neck RT by IMRT were evaluated for xerostomia. Informed written consent for IMRT

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Table 1. Patient and tumor characteristics

Age (years)	
Mean	57
Range	35–81
Sex	
Male	26
Female	7
Performance status	
0	22
1	10
PS2	1
Histology	
Squamous cell carcinoma	32
Non-Hodgkin lymphoma	1
Tumor site	
Nasopharynx	13
Oropharynx	10
Hypopharynx	10
Tumor stage*	
II	9
III	5
IV	19
Indication for RT	
Definitive	15
Post-operative	18
Concurrent chemotherapy	
Cisplatin (60–80 mg/m^2, 2–3 times)	10
Docetaxel (15 mg/m^2/week)	13
No chemotherapy	10

*UICC TNM classification, 6th edition 2002.

was obtained from all patients. Patient and tumor characteristics are shown in Table 1. Of the 33 patients, 13 had nasopharyngeal cancer, 10 had oropharyngeal cancers and 10 had hypopharyngeal cancers. Except for one non-Hodgkin's lymphoma of the nasopharynx, the remaining 32 tumors were squamous cell carcinomas.

For 17 patients with hypopharyngeal or oropharyngeal cancer, unilateral or bilateral neck dissection was performed before the start of IMRT. For one patient with tonsil cancer, simple tonsillectomy was performed without neck dissection. Of the 18 patients treated surgically before IMRT, 11 underwent laser coagulation of primary tumors, and four patients with tonsil cancer underwent simple tonsillectomy, only biopsy of primary tumors was done for the remaining three patients. No patients underwent potentially curative surgery or total laryngectomy before IMRT.

Twenty-three patients were treated with concurrent chemoradiation therapy. Concurrent cisplatin (60–80 mg/m^2, 2–3 times) was given to 10 patients with nasopharyngeal cancers, and weekly docetaxel (15 mg/m^2) was given to 13 patients with orohypopharyngeal cancers (10). No concurrent chemotherapy was given to one patient with malignant lymphoma, five patients of 70 years or older and four patients who refused chemotherapy.

Simulation and Treatment Planning

All patients were immobilized with a thermoplastic mask covering the head, neck and shoulders (Type-S thermoplastic based system, MED-TEC, Orange City, IA). Treatment-planning computed tomography (CT) scans were obtained with contrast medium at 5 mm slice intervals from the head through the aortic arch. For all patients, treatment-planning CT was done before IMRT (CT-1) and in the third or fourth week of IMRT for boost IMRT (CT-2).

Treatment planning for IMRT was done by inverse planning with commercial treatment-planning systems (Cadplan Helios,Varian Associates, Palo Alto, CA; Eclipse, Varian Medical Systems International Inc., Baden, Switzerland). The IMRT beam arrangements consisted of five or seven co-planar beams. Typically, seven beam angles of 60–75, 105–115, 135–150, 180, 210–225, 245–255 and 285–300° were used.

Target Definition and Dose Specification

Following the recommendations of the International Commission on Radiation Units report 50 and report 62 (11,12), the gross tumor volume (GTV) and the clinical target volume (CTV) were determined with axial CT images. For patients undergoing neck dissection or tonsillectomy before IMRT, the tumor bed of metastatic lymph nodes or primary tumors was regarded as high-risk CTV. For all patients, bilateral submandibular (level Ib) and jugular chain (level II–IV) lymph nodes were included in the initial CTV. Margins of 3–5 mm for treatment set-up and internal organ motion error were added to the CTV to determine the planning target volume (PTV). For planning organ at risk volume, a margin of 3 mm was added to the spinal cord. For the parotid glands, no margin was added in treatment planning. In the present analysis, all parotid glands were re-contoured by a single physician (Y.N.) to exclude observer variability on contouring the parotid glands, and the net volume and dosimetric parameters of the parotid glands could be determined with a re-calculated dose–volume histogram.

All patients were treated with whole-neck irradiation of 46–50 Gy in 23–25 fractions. The upper and middle regions of the neck were irradiated with IMRT based on CT-1, and the lower region of the neck was irradiated with the conventional anterior–posterior technique. After whole-neck irradiation, boost IMRT was given to the PTV for GTV and high-risk CTV on the basis of CT-2 to a total dose of 56–70 Gy in 28–35 fractions (median, 68 Gy). The daily prescribed dose to the PTV was 2.0 Gy.

The prescribed dose was normalized at a point in the PTV so that the 95% volume of the PTV received the prescribed dose