GPRSGGAQAKLGCCW) was found in L552S but not in other molecules. In contrast, the most common L552S epitope, peptide #29 (aa 141-160; IIPKEEHCKMPEAGEEQPQV), was found in three alternatively spliced forms of XAGE-1/CT12.1, i.e. L552S, XAGE-1, and -1b, but not in other human, animal, or bacterial molecules sequenced thus far.

**L552S-specific Abs preferentially recognize the C-terminal half of the protein**

As shown in Figure 4 (right panels), the peptide-based ELISA, but not the protein-based ELISA, was able to show not only major epitopes but also minor epitopes recognized by a relatively small proportion of antigen-specific Abs. In order to reveal the breadth of Ab responses that occurred in cancer patients, patient L552S-specific Abs were displayed on every peptide-coated plate. Seven patients' pleural effusions and sera positive for L552S protein-specific Abs were analyzed, and the epitopes thus defined are summarized in Table 3. The epitopes recognized by patient Abs frequently mapped to the C-terminal, but not to the N-terminal, of L552S. So far, only one patient (659-29, Figure 4, lower right panel) had Abs specific to peptides #4 and #5 located in the N-terminal portion. The C-terminal end of L552S was commonly and strongly recognized by all other specific Abs, whether the patients were from the USA or Japan. The minor epitopes of L552S-specific patient Abs were defined to lie in the region from peptide #17 to the C-terminal, which is identical to the open reading frame of the 3rd XAGE-1b isoform (21, 22, and Figure 6). This analysis of the L552S-specific Ab epitopes strongly suggests that patients were able to induce polyclonal Ab reponses recognizing multiple antigenic portions of L552S, and that these polyclonal responses easily skewed to single, epitope-specific responses.

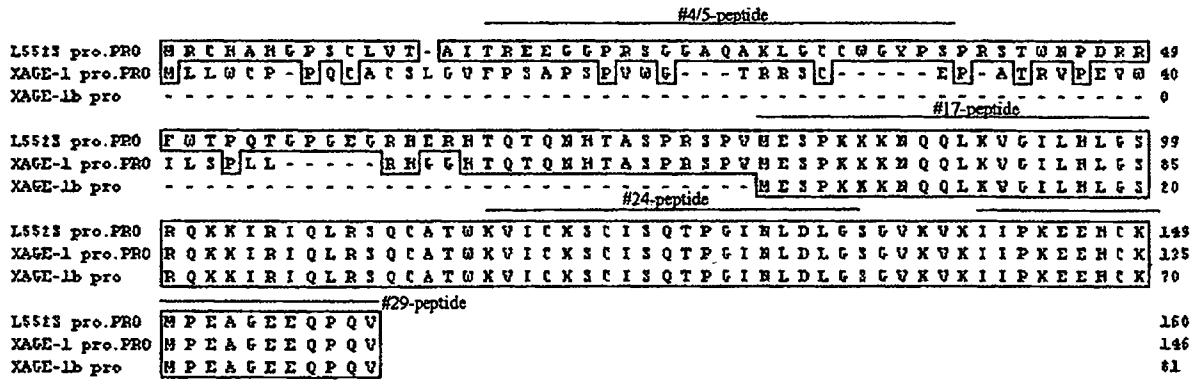
## Discussion

We have shown that lung cancer patients showed Ab responses specific to L514S and L552S in about 10% to 20% of the NSCLC cases studied from the USA and Japan. This high frequency of Ab responses specific to L552S and L514S, similar to that specific to NY-ESO-1, was seen in advanced cancer patients. The role that Abs specific to TAAs in the cytoplasm and nucleus play

**Table 3**
**Mapping of epitopes recognized by L552S-specific Abs from NSCLC patients**

| L552S Peptide | | Recognized by Patient Specimen | | | | | | | | Freq. |
|---|---|---|---|---|---|---|---|---|---|---|
| No. | AA Nos. | 298-19 | 659-29 | C361 | S105 | S109 | J9 | J46 | J56 | |
| #1 | 1-20 | | | | | | | | | - |
| #2 | 6-25 | | | | | | | | | - |
| #3 | 11-30 | | | | | | | | | - |
| #4 | 16-35 | | ++ | | | | | | | 14 |
| #5 | 21-40 | | ++ | | | | | | | 14 |
| #6 | 26-45 | | + | | | | | | | 14 |
| #7 | 31-50 | | | | | | | | | - |
| #8 | 36-55 | | | | | | | | | - |
| #9 | 41-60 | | | | | | | | | - |
| #10 | 46-65 | | | | | | | | | - |
| #11 | 51-70 | | | | | | | | | - |
| #12 | 56-75 | | | | | | | | | - |
| #13 | 61-80 | | | | | | | | | - |
| #14 | 66-85 | | | | | | | | | - |
| #15 | 71-90 | | | | | | | | | - |
| #16 | 76-95 | | | | | | | | | - |
| #17 | 81-100 | | | | + | + | | + | + | 43 |
| #18 | 86-105 | | | | | + | | + | | 29 |
| #19 | 91-110 | | | | | | | | | - |
| #20 | 96-115 | | | | | | | | | - |
| #21 | 101-120 | | | + | | | | | | 14 |
| #22 | 106-125 | | | | | | | | | - |
| #23 | 111-130 | | | | | | | | | - |
| #24 | 116-135 | + | | | | + | + | + | | 43 |
| #25 | 121-140 | | | | | + | + | + | | 33 |
| #26 | 126-145 | | | | | | | | | - |
| #27 | 131-150 | | | | | + | | | --- | 14 |
| #28 | 135-155 | + | | | | + | + | + | + | 57 |
| #29 | 141-160 | ++ | | -- | ++ | ++ | ++ | ++ | ++ | 71 |

## Figure 6




**Predicted amino acid sequences of the three isoforms, L552S, XAGE-1, and XAGE-1b.** Earlier reports predicted three potential open reading frames. L552S (160 aa), XAGE-1 (146 aa), and the shortest isoform XAGE-1b (81 aa) are shown here. Two major epitope regions (peptide #4/#5 and peptide #29) and two minor epitopes (peptide #17 and peptide #24) recognized by patient Abs are indicated by dotted lines.

in tumor immunity has not yet been discovered. Nonetheless, specific IgG responses helped by Th cells are considered to be, at the least, a suitable surrogate marker anticipating cellular responses, including CTLs. It has been shown that almost all patients positive for NY-ESO-1-specific Abs also had Th and/or CTL responses specific to NY-ESO-1, but Ab-negative patients did not have any of these cellular responses at all, even if their tumors expressed NY-ESO-1 mRNA (17, 18). Moreover, MAGE, TRP, and gp100 originally identified by patients' TILs/CTLs have been identified serologically as well (3, 4). It is likely that these cognate T and B cell responses specific to certain TAAs occur frequently in cancer patients because intracellular TAAs overexpressed in tumors may be cross-primed through necrosis/apoptosis of tumor cells with professional APCs, or through the nonspecific inflammation that surrounds tumors, resulting in the induction of broad immunity, including Ab and CTL responses (22). In the advanced stages of lung cancer, LPEs frequently include T cells and B cells that are potentially specific to TAAs. The data reported here also show that Ab responses specific to TAAs existed in pleural effusions with infiltrating lymphocytes, as described recently (23). Interestingly, patient LPE 298-19 was found to have Abs specific to L552S and L514S, at different titers. Other patients had similar titers of both L552S- and NY-ESO-1-specific Abs (data not shown). These humoral immune responses to multiple TAAs seem to occur frequently in cancer patients.

This report explores the B cell/Ab epitopes predicted for TAAs L514S, L552S, and XAGE in several patients. L552S and XAGE-1 are alternatively spliced forms of each other. In Figure 3 we showed that one patient's Abs recognized peptide #4/#5 of L552S. Even though we have not confirmed L552S expression in this particular patient, this demonstrates that the tumor expressed the protein, including at least peptide #4/#5 of L552S, because this epitope does not have any homology to other human protein sequences. Molecular analyses and amino acid alignment of L552S also showed that only L552S, and not XAGE-1 or XAGE-1b, was the predicted protein containing peptide #4/#5 of L552S, as shown in Figure 6. This process, combined with both the epitope analysis and the homology search, is very useful for detecting and confirming humoral responses specific to TAAs in cancer patients and can also be used to assess cellular responses. The sequential analyses conducted in this report clearly demonstrate that L514S and L552S, which are overexpressed at the mRNA level in NSCLC, were recognized by specific Abs from lung cancer patients. By analogy we show that L514S and L552S are two of the most immunogenic TAAs studied in lung cancer, since NY-ESO-1 is one of most immunogenic CT antigens studied to date.

The peptide-based ELISA was able to identify minor epitopes recognized by L552S-specific Abs. This is interesting, as it may relate to epitope spreading (24, 25), and it is also quite interesting in terms of finding minor epitopes of TAAs in cancer patients. Pleural effusion 298-19 had Abs specific to L552S peptides #29 and #24. Another patient's serum specimen (J9) also had Abs specific to peptide #24, as well as Abs specific to peptide #29. Furthermore, these peptide #24-specific Ab responses were not observed in the sera of normal volunteers, nor in that of patients for which no L552S-specific Abs were detectable (data not shown). However, the Western band recognized by the L552S-specific Abs of these patients' specimens could not be cleared by peptide #24 without #29 peptide, suggesting that peptide #24 is one of the minor epitopes. It is also likely that peptides #17 and #21, which are weakly recognized by patient Abs, are also minor epitopes (Table 3). These data show that Ab responses specific to L552S have spread from one major immunogenic epitope to multiple minor epitopes. Kinetic analyses of these major and minor epitopes in cancer patients could reveal how epitope spreading and/or skewing takes place during disease progression and regression.

In Figure 6, the sequence alignment of all three alternatively spliced forms, L552S, XAGE-1, and XAGE-1b, is shown. The shortest, XAGE-1b, is an 81-aa protein which extends from peptide #17 to the C-terminal peptide #29 of L552S (21). The epitope analysis, including the presence of minor Ab epitopes, suggests that the protein expressed is mainly the 3rd isoform XAGE-1b, and not L552S or XAGE-1a. This preferential recognition by patient Abs of the C-terminal half and not the N-terminal half was also observed among Japanese patients whose Abs were specific to L552S (Table 3). Moreover, previous data from our own and other groups showed that mRNA expression of both L552S and XAGE-1 using specific primers is much lower than that of the common portion shared between L552S, XAGE-1a and the 3rd short isoform XAGE-1b (16, 21). A recent report also observed that mRNA expression of either XAGE-1 or L552S (XAGE-1c) isoform is rare, but that XAGE-1b (26) is abundantly expressed. On the other hand, it has also been observed that the major epitope of polyclonal L552S Abs generated in L552S-immunized rabbit is skewed to the C-terminal end of the protein, even though minor epitopes exist in the N-terminal half of L552S. These data suggest that the preference of L552S-specific Abs in NSCLC patients for certain epitopes is mainly caused by the expression of the shortest isoform XAGE-1b of the three, and by the existence of an immunogenic amino acid sequence in L552S.

The proof and characterization of the preexisting, high-titer Ab responses specific to the novel CT antigens L514S and L552S have been compared to that of the known CT antigen NY-ESO-1. We deduced that both predicted CT antigens are expressed in the tumor site at the protein level, as the epitopes identified were recognized by specific Abs and have no sequence homology to other known molecules in the Entrez protein database. As these specific IgG responses are likely to be mediated by cognate T cell responses (24, 27), studies are in progress to determine whether specific T cell responses, including CTL responses, are present in Ab-positive and -negative patients.

## Abbreviations

CT, cancer-testis; LPE, lung pleural effusion; NFDM, non-fat dry milk; NSCLC, non-small cell lung cancer; TAA, tumor-associated antigen

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## Materials and methods

**Pleural effusions and sera of lung cancer patients**

All pleural effusions were collected from advanced stage lung cancer patients (stage IV) according to Internal Review Board

guidelines. Fluid was centrifuged for 15 min at 300 x *g* to remove mononuclear cells. Supernatant was decanted into a sterile tube, passed through a 5 μM filter, and stored frozen at -20°C. Sera were obtained from NSCLC patients (stages I-IV) (Samplex Inc., Westlake Village, CA, USA and Lifeblood Biological Services, Memphis, TN, USA) and from normal donors with informed consent. Sera and pleural effusions were used at dilutions of 1:50 to 1:7 x $10^6$.

#### Quantitative real-time RT-PCR analysis

To compare the relative level of gene expression in multiple tissue samples, a panel of 66 cDNA samples was constructed using total RNA extracted from tissues and/or cell lines. Real-time PCR was performed using L514S-specific primers to quantify the copy number in each cDNA sample. Each cDNA sample was tested twice, and each reaction was reported as an average of the copy number of the gene of interest normalized against the average of the actin copy number in each cDNA sample. All RT-PCR reactions were performed on an ABI PRISM 7700 Detector (PE Biosystems, Foster City, CA, USA). The details as to how the RT-PCR was performed have been published previously (15, 16).

#### Recombinant TAA proteins and peptides

L514S was PCR-amplified with the following phosphorylated primer pair: 5'-CACACTAGTGTCCGCGTG GCGGCCTAC-3' and 5'-CATGAGAATTCATCACATGCCC TTGAAGGCTCCC-3', as described in an earlier report (15). The phosphorylated PCR product was digested with *Eco*RI and ligated into a modified pET28 vector (Novagen, Madison, WI, USA) containing a His tag in-frame at the N-terminus. The ligated DNA was transformed into Top10 cells (Invitrogen, Carlsbad, CA, USA) and minipreps screened through DNA sequence analysis. The correct clone was then transformed into BL21 (DE3) CodonPlus RIL cells (Stratagene, La Jolla, CA, USA). An overnight culture was started from an isolated colony grown in LB plus kanamycin (30 μg/ml) and chloramphenicol (34 μg/ml). One-liter volume day cultures were grown in 2-L baffled flasks using 2xYT media with the same antibiotics. The cultures were grown to an $OD_{600}$ of 0.4-0.6 and induced with 1 ml of 1 M IPTG. The cultures were grown another 3 h and then harvested. The pellets were collected and washed with 1xPBS and then resuspended in lysis buffer (20 mM Tris, pH 8.0, 500 mM NaCl), and frozen at -20°C overnight. The frozen pellets were then thawed and lysed through a French press and centrifuged at high speed. The inclusion body pellet was then washed with 0.5% CHAPS, 20 mM Tris (pH 8.0), 500 mM NaCl, followed by resuspension in 8 M urea, 20 mM Tris (pH 8.0), 500 mM NaCl. The denatured pellet was then passed over a nickel column and eluted with increasing concentrations of imidazole in 20 mM Tris (8.0), 8 M urea, 100 mM NaCl buffer. The elution fractions that contained protein were dialyzed into 10 mM Tris (pH 8.0).

The L552S coding region was also PCR-amplified with the following primers, 5'-CGGTGCCACGCCCATGG ACCTTC-3' and 5'-CTGAGAATTCATTAAACTTGTGGTTG CTCTTCACC-3' as described in a previous report (16). The PCR product was digested with *Eco*RI and cloned into a modified pET28 vector that had been cut with *Eco*72I and *Eco*RI. Once the sequence was confirmed, the construct was transformed into BL21 pLys S and BL21 CodonPlus cells. Protein purification was performed as described above.

The NY-ESO-1 coding region was PCR-amplified, and the recombinant protein purified as described in an earlier report (20).

L514S and L552S peptides, 20 aa in length and which overlap by 15 aa, were synthesized by solid phase Fmoc synthesis. Their purity was determined by HPLC. Individual peptides were dissolved in DMSO at a concentration of 10 mg/ml and stored at -20° before use.

#### Protein- and peptide-based ELISAs

For the protein-based ELISA, 50 μl of each protein solution (10 pmole/ml) was added to each well of a 96-well plate (Nunc, Denmark) with bicarbonate coating buffer (pH 9.5) and incubated overnight at 4°C. For the peptide-based ELISA, 50 μl of pooled or individual peptide solution (20 μg/ml) was coated in the same manner. The protein or peptide solution was flicked out, and the plates were blocked with 10% NFDM-containing PBS for at least 3 h at room temperature. The plates were then washed with 0.05% Tween-containing PBS and the samples (50 μl/well), that is the patients' pleural effusions and sera serially diluted with 5% goat sera and 5% NFDM-containing PBS, were added to the plates which were then incubated for 3 h at room temperature or overnight at 4°C. Following washing with PBS-Tween, diluted goat antihuman IgG conjugated to HRP (Jackson Laboratories, Bar Harbor, ME, USA) was added to the plates which were then incubated 30 min at room temperature. After washing, substrate solution (TMS ELA substrate, Bio-Rad Laboratories, Hercules, CA, USA) was added and the plates incubated 15 min at room temperature. After adding stop solution (0.1 N sulfuric acid solution), the absorption at 450 nm relative to the reference absorption at 560 nm was determined with a spectrophotometer. The presence of Ab was defined as a signal/noise (S/N) ratio >3; L514S, L552S, and NY-ESO-1 served as reference antigens for each other.

#### Western blot analysis

Patient pleural effusions and sera antibody responses against L514S, L552S, and NY-ESO-1 were tested. Purified recombinant L514S, L552S, and NY-ESO-1 proteins (200 ng) were loaded in SDS-PAGE gel and electrophoresed. The gels were blotted onto nitrocellulose membranes. After blocking the membranes with 10% nonfat dry milk (BioRad)-containing PBS, the diluted sera (1:300 to 1:30,000 dilution) or pleural effusions (1:30 to 1:3,000 dilution) were added to the membranes and incubated for 3 h at room temperature or overnight at 4°C. The nitrocellulose membranes were then incubated with HRP-conjugated antihuman IgG (Jackson Laboratories, Bar Harbor, ME, USA) and visualized using a chemoluminescent substrate (ECL™, Amersham Pharmacia Biotech, Uppsala, Sweden). The presence of specific Abs was defined as distinct Western bands with the sample dilutions described. L514S, L552S, and NY-ESO-1 were used as reference antigens for each other.

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# Clinical development of EGFR-tyrosine kinase inhibitors in Japan

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**Abstract** Although the initial impact of the epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) gefitinib may have been less than spectacular in the field of non-small cell lung cancer (NSCLC), this EGFR-TKI does offer a therapy that, at least in the short term, markedly reduces tumors without bone marrow suppression including neutropenia and without causing severe nausea and vomiting even in NSCLC patients with the worst prognosis. This raises the possibility of putting the disease under control if only temporarily. Now we must be aware that overcoming gene mutation in lung cancer is the next significant milestone for new therapeutics. This report discusses clinical trials of EGFR-TKIs focusing on Japanese contributions to current knowledge, *EGFR* mutation, and future directions. A Japanese phase I clinical trial saw the first super-responders to gefitinib. Two randomized phase II trials identified Japanese, females, and those with adenocarcinoma of the lung as specific populations sensitive to gefitinib. Unexpectedly, in the context of first-line chemotherapy four phase III trials gave completely negative results for additional clinical benefit by EGFR-TKIs combined with standard chemotherapy. However, subset analysis suggested efficacy of this treatment strategy in nonsmokers and patients harboring activated-type *EGFR* mutations. In the settings of second-line and later therapy, two independent randomized placebo-controlled trials, BR.21 with erlotinib and ISEL with gefitinib, revealed better duration of overall survival, time to progression, and response rate in the EGFR-TKI versus control groups, although the result was nonsignificant in the latter study. Data suggesting that adenocarcinoma, Asian race, female, and nonsmoker are associated with better response to EGFR-TKI may be closely related with phenotype of *EGFR* mutations, making this parameter a "response predictive marker." On the other hand, some reports have stated that gene amplification of *EGFR* by FISH analysis shows better correlation with clinical benefit of EGFR-TKIs than that assessed by other means in large-scale phase III trials (BR21 and ISEL). Further validation of response predictive markers is needed. Recent studies of EGFR-TKIs in NSCLC provide novel biological insights and have given birth to the concept of patient selection for this disease. Further investigation of the biological significance of *EGFR* mutation and its validation as response predictive marker will lead to better treatments to come for NSCLC.

**Keywords** EGFR-TKI · Gefitinib · Erlotinib · *EGFR* mutation

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## Introduction

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have been clinically available for the treatment of nonsmall cell lung cancer

(NSCLC) for the past 4 years. In the course of clinical development of EGFR-TKIs, in comparison with conventional anticancer agents many unexpected findings were observed such as relating to tumor shrinkage, specific responder subsets, adenocarcinomatous disease, and gene mutation. Hence although knowledge concerning EGFR-TKIs and *EGFR* gene mutation is advancing in the laboratory setting, clinically it is unclear how we should use EGFR-TKIs in NSCLC and which patients might benefit most from these agents. In this review, clinical trials of EGFR-TKIs are recounted and a key factor for drug sensitivity, *EGFR* mutation, is discussed.

## Clinical trials of EGFR-TKIs

Four phase I trials of EGFR-TKI including one Japanese study were performed in a total of 254 patients [4, 8]. These trials defined diarrhea and liver function test abnormality as dose-limiting factors. Five of 23 patients demonstrated partial responses (PRs) without dose–response tendency (Table 1). Toxicity profiles were quite different to those commonly observed with conventional anticancer agents. Ten percent of patients failed treatment at doses >600 mg/day and these early studies could not identify an optimal dosing schedule. Based on the results of phase I, the phase II IDEAL1 study was conducted in 210 previously treated advanced NSCLC patients in Japan, Australia, and Europe [1]. In this large-scale international study, a similar objective tumor response rate (20%) to those of previous studies was observed. There was no difference of clinical response between patients receiving 250 mg/day and those on 500 mg/day, whereas toxicity was more severe in the higher-dose group. Subset analysis revealed startling clinico-pathological subpopulations with especially high drug sensitivity to EGFR-TKI namely Japanese patients, females, nonsmokers, and those with adenocarcinoma (Table 2). In particular, Japanese females exhibited an overall response rate >50% in this analysis. For the first time, unlike conventional anticancer agents these results suggested that EGFR-TKIs are efficacious in specific subpopulations. While that phase II trial was ongoing, two large phase III trials in untreated NSCLC were begun in the USA and Europe [2, 3]. The rationale of these two clinical trials, INTACT1 and INTACT2, was based on preclinical studies that suggested synergistic effects of taxane plus gefitinib against cancer cells in vitro and in vivo. Hence, gefitinib or placebo was added onto standard chemotherapy regimens cisplatin/gemcitabine (INTACT1) and carboplatin/paclitaxel (INTACT2) [2, 3]. Both trials showed that there was no evidence for prolonged survival time with add-on gefitinib for either standard chemotherapy schedule. The same negative result was observed in another phase III trial using the same design with erlotinib as well as gefitinib [5]. However, in this trial subset analysis suggested enhanced efficacy of EGFR-TKI therapy among nonsmokers and those harboring activated-type *EGFR* mutations. Two subsequent studies of second-line and later treatment, BR.21 and ISEL, gave conflicting results for overall survival time, time to progression, and response rate: the former suggested additional benefit of add-on EGFR-TKI and the latter gave negative results [10, 11].

**Table 1** Antitumor activity of gefitinib in Japanese phase I study

| | Total | PR (%) |
|---|---|---|
| All cases | 31 | 5 (16) |
| NSCLC | 23 | 5 (22) |
| Histology | | |
| Adenocarcinoma | 19 | 5 (26) |
| Squamous cell carcinoma | 4 | 0 (0) |
| Gender | | |
| Male | 15 | 1 (7) |
| Female | 8 | 4 (50) |

*PR* partial response

To clarify the clinical benefit of EGFR-TKIs in *EGFR* mutation-positive NSCLC, prospective phase II (WJTOG0403) and phase III (WJTOG3405) studies are now underway (Fig. 1). The results of these investigations aim to give us data that will enable us better to understand *EGFR* mutational status and whether mutant EGFR phenotype confers clinical benefit in patients.

## *EGFR* mutation and drug sensitivity

To use gefitinib effectively in clinical settings we must first identify patient populations who respond well to this agent. As mentioned above, data from IDEAL1 revealed that gefitinib is highly effective in Japanese, females, adenocarcinomatous histology, good performance status (PS), and nonsmokers (Table 2). Since the target molecule of EGFR-TKIs is EGFR, some correlation between expression patterns of EGFR protein and clinical outcome was widely speculated. However, IDEAL1 and 2 found no correlation between these parameters clinically, questioning the concept of molecular-targeting drugs. However, the answer to this question was provided by the striking findings regarding *EGFR* gene mutations [7, 9]. These *EGFR* mutations, located on the ATP binding site (exon 19–21) of

**Table 2** Overall survival by patient characteristics: IDEAL1

| Characteristic | Evaluable (*n*) | MST, days (95%CI) | *P*-value[a] | ORR, % (*n*) |
|---|---|---|---|---|
| All patients | 209 | 241 (205–276) | | 18.7 (39/208) |
| Dose | | | | |
| 250 mg/day | 103 | 232 (161–318) | 0.716 | 18.4 (19/103) |
| 500 mg/day | 106 | 243 (203–309) | | 19.0 (20/105) |
| Age | | | | |
| <65 years | 145 | 238 (198–284) | 0.5598 | 19.4 (28/144) |
| >65 years | 64 | 241 (188–371) | | 17.2 (11/64) |
| Gender | | | | |
| Female | 61 | 397 (261–439) | 0.0025 | 34.4 (21/61) |
| Male | 148 | 212 (161–243) | | 12.2 (18/147) |
| WHO PS | | | | |
| 0–1 | 182 | 268 (234–318) | <0.0001 | 21.0 (38/181) |
| 2 | 27 | 83 (57–121) | | 3.7 (1/27) |
| Histology | | | | |
| Adenocarcinoma | 131 | 300 (236–371) | <0.0001 | 26.0 (34/131) |
| Other | 78 | 198 (129–232) | | 6.5 (5/77) |
| Smoking history | | | | |
| Yes | 104 | 186 (127–241) | <0.0001 | 12.5 (13/104) |
| No | 53 | 414 (357–534) | | 37.7 (20/53) |

*MST* mean survival time, *ORR* overall response rate

[a] Log-rank test

EGFR tyrosine kinase domain, are missense or deletion mutations causing substitution or partial deficiency of amino acid. Based on the results of basic studies, structural changes of the ATP binding site were found to increase binding affinity for ATP and gefitinib. In other words, under physiological conditions *EGFR* mutations are activating mutations that constitutively increase tyrosine kinase activity, and it is speculated that signals via EGFR are thereby abnormally enhanced and have greater impact on malignant transformation such as cancer cell proliferation. Fortunately, since these mutations are thought to have more highly augmented binding affinity for gefitinib than for ATP, they may display overwhelmingly high sensitivity induced by EGFR-TKIs. What is surprising is the correlation between frequency of *EGFR* mutations and clinical antitumor effects. We compared mutation rates and projected response rates obtained from IDEAL 1 and 2 and from 154 subjects in the clinical study in which our institute participated, and found that the *EGFR* mutation was highly correlated with clinical response (Table 3). In addition, it was reported at the American Society of Clinical Oncology (ASCO) meeting 2005 that *EGFR* gene mutation is closely related to

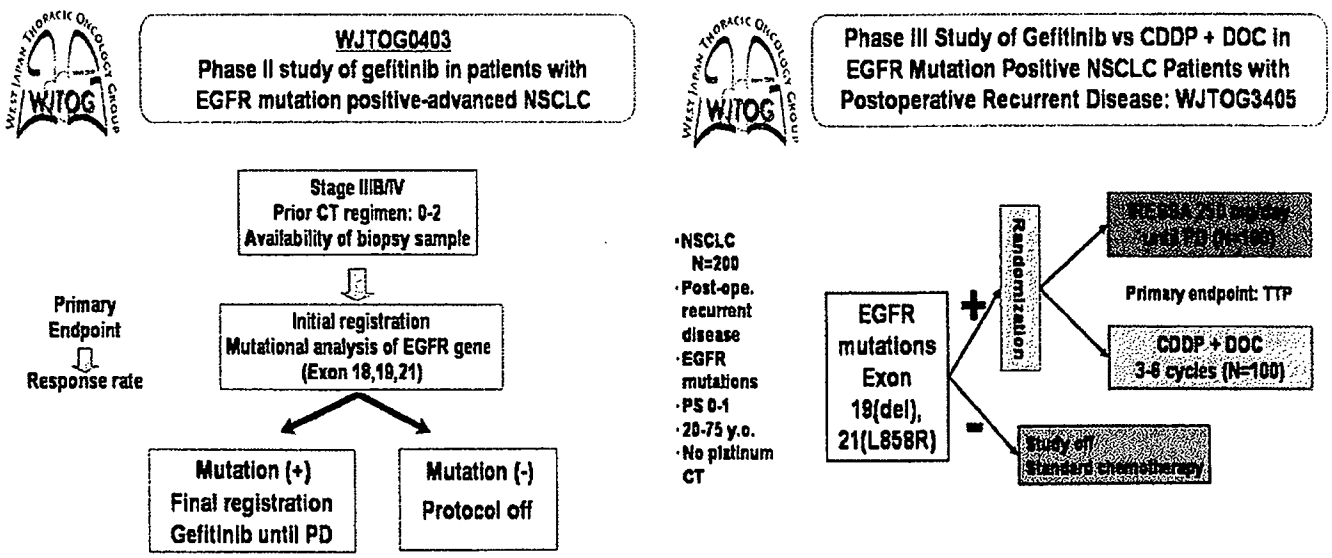


**Fig. 1** Trial design of two ongoing prospective phase II (WJTOG0403) and phase III (WJTOG3405) studies investigating clinical benefit of EGFR-TKIs in *EGFR* mutation-positive NSCLC

**Table 3** Estimated response rate (RR) for gefitinib and *EGFR* mutation in patients with NSCLC

| Patient population | Estimated RR (%) | *EGFR* mutation (%) | |
|---|---|---|---|
| | | Guillermo | Mitsudomi |
| Euro-American | 10 | 2 | – |
| Japanese | 28 | 26 | 40 |
| Japanese-adenocarcinoma | 35 | 32 | 49 |
| Female Japanese-adenocarcinoma | 50 | 57 | 62 |

gefitinib sensitivity [6]. It is thought that the reason for the high response rate associated with Japanese race, female, adenocarcinoma, good PS, and nonsmokers is high frequency of *EGFR* mutations in these populations.

## Future challenges

To establish clinical usage of EGFR-TKIs there are many issues to be addressed such as: (1) precisely identifying the site of *EGFR* mutations associated with drug sensitivity; (2) conducting a prospective clinical study of *EGFR* mutation and drug sensitivity; (3) establishing techniques to detect *EGFR* mutation precisely; (4) investigating efficacy of EGFR-TKI therapy in patients without *EGFR* mutations; (5) identifying patients responsive to EGFR-TKIs among those without *EGFR* mutations and clarifying the mechanism of action of EGFR-TKIs; and (6) clarifying mechanisms of EGFR-TKI resistance and developing drugs to overcome this resistance.

## Combined use with conventional anticancer agents

Currently, gefitinib is the only EGFR-TKI available in Japan. How should we use gefitinib in combination with other anticancer agents? Large-scale clinical studies in Caucasian NSCLC patients indubitably have shown that concomitant use of conventional anticancer agents and gefitinib has no clinical usefulness in that patient population. Considering the association between gefitinib sensitivity and *EGFR* gene mutations, however, it seems too early to make a similar conclusion in Japanese patients in whom *EGFR* gene mutations might be more frequent. Therefore, it is important clinically to test gefitinib in Japanese patients concomitantly taking conventional anticancer drugs. In addition, in the context of combination therapeutic regimens not only simultaneous administration with conventional anticancer agents but sequential and maintenance therapies should be evaluated. To this end, the WJTOG phase III clinical trial is currently ongoing. Patients enrolled in this trial are divided into two groups: those taking three courses of two chemotherapeutic agents including one platinum-based drug followed by three courses of gefitinib, and the group on six courses of two drugs including one platinum drug alone. This trial, expected to terminate in April 2005, is aimed to show conclusively whether serial/sequential gefitinib therapy is useful in Japanese patients with NSCLC.

## Conclusions

The advent of EGFR-TKIs convinced us that biological study of these agents in NSCLC could improve prognosis of these patients. Although the improvement elicited by gefitinib may be small so far, this agent does at least provide a new form of therapy that over the short term leads to markedly reduced tumor size without bone marrow suppression including neutropenia and no severe nausea and vomiting even in those patients with the worst prognosis. This raises the possibility of placing this rapidly fatal disease under some control. Doctors must be aware that making inroads towards understanding the implications of gene mutation in lung cancer will be a milestone for new therapeutics.

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# Common arm analysis: One approach to develop the basis for global standardization in clinical trials of non-small cell lung cancer

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**Summary** The global development of new anticancer treatments is desirable. However, whether results of clinical trials performed in one population can be fully extrapolated to another population remains in question. We retrospectively compared "common arms" of platinum-based doublet phase III trials among Japanese, European, and American patients with non-small cell lung cancer to develop the basis for global standardization in clinical trials. Patient demographics were very similar through all studies, indicating that extrinsic ethnic factors including socioeconomic factors, medical service background, and patient selection process for clinical trials may be consistent between geographically different oncology groups. The doses of docetaxel, gemcitabine, and vinorelbine were lower in Japanese studies. The toxicity profile was generally acceptable and similar among many studies. Thus, the dose and schedule of anticancer agents established in prior phase I and II studies conducted in each country were appropriate and applicable to large patient populations in these countries. Response rates seemed to be distributed randomly from one study to another, whereas patient survival might be better in Japanese studies. In conclusion, geographical differences in the dose of anticancer agents, response, survival and toxicity of lung cancer chemotherapy were actually observed. However, extrapolation of clinical data obtained in one country to another population and global clinical trials were considered possible with adequate dose adjustment based on dose finding studies using a carefully projected protocol.


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## 1. Introduction

Lung cancer is one of the most common malignancies and the leading cause of cancer-related deaths in many countries. In the year 2000, the annual number of deaths from lung cancer was estimated to be 1.1 million worldwide, and the incidence lung caner is increasing globally at a rate of 0.5% per year [1]. Lung cancer currently claims more than 55 000 lives annually in Japan, and this figure is projected to double during the next three decades due to the aging of the Japanese population [2]. Non-small cell lung cancer (NSCLC) comprises 80% of all lung cancers, and more than half of the patients with this disease are found to have developed distant metastases or pleural effusion at the time of the initial diagnosis. These patients can be treated with systemic chemotherapy, but the efficacy of currently available anticancer agents is limited to the extent that patients with advanced disease rarely livelong [3].

The development of new anticancer agents and chemotherapeutic regimens are among the urgent tasks for medical oncologists who are involved in the treatment of lung cancer. Since it is time- and money-consuming work, the development of new agents and regimens is desirable on a global scale. Under the present situation in Japan, in that we are considerably behind with the development of new anticancer agents, it is worth evaluating the possibility that the results of clinical trials held outside Japan could be used for approval of these agents by the Japanese authorities. However, whether the results of clinical trials performed in one population can be fully extrapolated to another population remains in question due to the potential differences in trial designs, study-specific criteria, patient demographics, and population-related pharmacogenomics. According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guideline E5, Ethnic Factors in the Acceptability of Foreign Clinical Data, the impact of genetic and physiologic (intrinsic) factors and cultural and environmental (extrinsic) factors upon the efficacy and safety of anticancer agents at a particular dosage and dose regimen must be assessed for the application of new agent approval [4].

One approach to develop the basis for global standardization in clinical trials of anti-NSCLC agents is a planned comparative analysis of a "common arm" with similar eligibility, staging, response and toxicity criteria of prospectively designed and conducted separate phase III trials for the treatment of advanced NSCLC, although this approach may have potential limitation in comparability [5]. In this review we retrospectively compared the outcome of phase III trials conducted in Japan, Europe, and USA for chemotherapy doublet regimens using a platinum and a third-generation cytotoxic agent, including paclitaxel, docetaxel, gemcitabine, and vinorelbine.

## 2. Methods

Combinations of paclitaxel and carboplatin, docetaxel and cisplatin, gemcitabine and cisplatin, and vinorelbine and cisplatin were evaluated in patients with advanced NSCLC as the post-marketing sponsored phase III trials in Japan [6,7]. Phase III trials evaluating these regimens conducted outside Japan were identified by Medline searches. The selection criteria of phase III trials for this analysis were (1) first-line treatment for stage IIIB or IV NSCLC; (2) not intended for a special cohort of patients such as the elderly or those with poor performance status; (3) each arm included more than 120 patients; (4) tumor response was evaluated according to the World Health Organization (WHO) criteria, modified WHO criteria such as Eastern Cooperative Oncology Group (ECOG) criteria and Southwest Oncology Group (SWOG) criteria, or response evaluation criteria in solid tumors (RECIST) criteria; (5) toxicity was evaluated according to the WHO criteria or the National Cancer Institute-Common Toxicity Criteria (NCI-CTC). The dose and schedule of anticancer agents, patient demographics, treatment delivery, tumor response, patient survival, and toxicity were compared between common arms in separate phase III trials. To assess the influence of demographic variables on tumor response and survival, multiple linear regression analysis was performed as previously described [8].

## 3. Results

### 3.1. Taxane and platinum

The schedule was identical between the studies in both paclitaxel and carboplatin, and docetaxel and cisplatin combinations (Tables 1 and 2). The dose of paclitaxel ranged from 175 to 225 mg/m$^2$ without ethnic tendency. The dose of docetaxel was set to be 20% lower in a Japanese study [7] than that of USA studies [9,10]. This difference was mainly attributable to differences in the criteria of the maximum tolerated dose in phase I studies of docetaxel between Japan and the USA. Patient demographics were very similar among these studies. Response rates (RRs) in the combination of paclitaxel and carboplatin varied widely from 17% to 46%, and median survival time (MST) from 7.8 to 12.3 months. The RR and MST in Japanese and Greek studies appeared to be better than those in ECOG study, but did not differ from those in other American studies. A multiple linear regression analysis failed to show correlation between demographic variables and the RR or MST. In the docetaxel and cisplatin combination, the RR and survival in the Japanese study appeared to be better that those in the ECOG study [9], but similar to those in the other USA study [10].

Among paclitaxel and carboplatin studies, the incidence of grade 3-4 neutropenia and febrile neutropenia was higher in the Japanese study than in the other studies. The toxicity profile of the docetaxel and cisplatin combination was identical among all studies.

### 3.2. Gemcitabine and cisplatin

The dose of gemcitabine per one course was smaller in the Japanese study than in other studies outside Japan (Table 3). The RR in ECOG study was lower than that in European studies, while the MST of 14.8 months and 1-year survival rate of 60% in the Japanese study seemed higher than those in the other studies [6]. There was no correlation between demographic variables and the RR or MST in a multiple linear regression analysis.

Table 1 The combination of carboplatin and paclitaxel

| Characteristics | Japan [6] | Greece [13] | Greece [14] | EU [18] | ECOG [19] | SWOG [19] | SWOG [5] | USA [20] | USA [12] |
|---|---|---|---|---|---|---|---|---|---|
| Chemotherapy dose | | | | | | | | | |
| CBDCA (AUC) | 6 (day 1) | 6 (day 1) | 6 (day 1) | 6 (day 1) | 6 (day 1) | 6 (day 1) | 6 (day 1) | 6 (day 1) | 6 (day 1) |
| PTX (mg/m$^2$) | 200 (day 1) | 175 (day 1) | 200 (day 1) | 200 (day 1) | 225 (day 1) | 225 (day 1) | 225 (day 1) | 225 (day 1) | 225 (day 1) |
| Demographics (% not specified) | | | | | | | | | |
| No. of patients | 145 | 185 | 252 | 309 | 290 | 206 | 182 | 190 | 345 |
| Age (median) (range) | 63 (33–74) | 65 (30–83) | 63 (31–81) | 58 (27–76) | 63 (30–85) | 62 (26–80) | 63 (28–80) | 62 (28–80) | 63 (31–85) |
| Female | 32 | 14 | 13 | 17 | 38 | 30 | 37 | 34 | 39 |
| PS 0–1 | 100 | 80 | 86 | 83 | 95 | 100 | 100 | NA | 91 |
| Stage IV | 81 | 49 | 62 | 68 | 86 | 88 | 87 | 77 | 78 |
| Non-squamous | 79 | 63 | 69 | 63 | NA | NA | 82 | NA | 81 |
| Treatment delivery and efficacy (% not specified) | | | | | | | | | |
| Cycles (median) | 3 | NA | NA | 4 | 4 | NA | 4 | NA | 6 |
| Response rate (95% CI) | 32 (25–40) | 46 (39–53) | 28 (22–34) | 23 (20–30) | 17 (13–21) | 25 (19–31) | 34 (27–41) | 23 (17–29) | 29 (24–34) |
| MST (month) (95% CI (month)) | 12.3 (NA) | 11.0 (10–12) | 10.4 (8.8–12) | 8.2 (7.4–9.6) | 8.1 (7.0–9.5) | 8.6 (7.2–10.7) | 9.0 (NA) | 7.8 (NA) | 9.9 (NA) |
| 1-year survival (%) | 51 | 43 | 42 | 32 | 34 | 38 | 37 | 32 | 42 |
| Grade 3-4 toxicity (%) | | | | | | | | | |
| Neutropenia | 88 | 14 | 15 | 51 | 63 | 57 | NA | 65 | 6 |
| Febrile neutropenia | 16 | 9 | 0 | 4 | 4 | 2 | 3 | NA | NA |
| Thrombocytopenia | 11 | 2 | 2 | 2 | 10 | 10 | 8 | 8 | NA |
| Neuropathy | 5 | 26 | 8 | 9 | 10 | 13 | 16 | 5 | 1 |

Table 2 The combination of cisplatin and docetaxel

| Characteristics | Japan [7] | ECOG [9] | USA [10] |
|---|---|---|---|
| Chemotherapy dose | | | |
| CDDP (mg/m$^2$) | 80 (day 1) | 75 (day 1) | 75 (day 1) |
| DTX (mg/m$^2$) | 60 (day 1) | 75 (day 1) | 75 (day 1) |
| Demographics (% not specified) | | | |
| No. of patients | 151 | 289 | 408 |
| Age (median) (range) | 63 (30–74) | 63 (34–84) | 61 (30–81) |
| Female | 36 | 37 | 28 |
| PS 0–1 | 96 | 94 | 96 |
| Stage IV | 100 | 86 | 67 |
| Non-squamous | 89 | NA | 68 |
| Treatment delivery and efficacy (% not specified) | | | |
| Cycles (median) | 3 | 4 | 5 |
| Response rate (95% CI) | 37 (29–45) | 17 (12–21) | 32 (27–36) |
| MST (month) (95% CI (month)) | 11.3 (NA) | 7.4 (6.6–8.8) | 11.3 (10.1–12.4) |
| 1-year survival | 48 | 31 | 46 |
| Grade 3-4 toxicity (%) | | | |
| Neutropenia | 74 | 69 | 75 |
| Febrile neutropenia | 2 | 11 | 5 |
| Thrombocytopenia | 1 | 3 | 3 |
| Neuropathy | 0 | 5 | 4 |

The toxicity was similar among many studies except for the gemcitabine and cisplatin arm of the Iressa NSCLC Trial Assessing Combination Treatment (INTACT) study [11], where the incidence of grade 3-4 neutropenia and thrombocytopenia was reported to be about one tenth of that in other studies (Table 3).

### 3.3. Vinorelbine and cisplatin

The dose of vinorelbine per one course was also smaller in the Japanese study than in other studies outside Japan (Table 4). The RR in the Greek study was higher than that in an American study. There was no difference in survival for this combination among all studies. There was no correlation between demographic variables and the RR or MST in a multiple linear regression analysis.

Grade 3-4 neutropenia was less common in the Greek study than in other studies, but the frequency of febrile neutropenia in that study was intermediate among studies.

## 4. Discussion

This study showed that geographical differences in the outcome of lung cancer chemotherapy may be present. However, extrapolation of clinical data in a country to another population and global clinical trials were considered possible with adequate considerations as discussed below.

The dose of third-generation cytotoxic agents was smaller in Japanese studies than in European and American studies. The toxicity profile was generally acceptable and similar among many studies. Thus, the dose and schedule of anticancer agents established in prior phase I and II studies conducted in each country were appropriate and applicable to large patient populations of these countries. Patient demographics were very similar through all studies, indicating that extrinsic ethnic factors may be comparable and consistent between geographically different oncology groups. These factors include socioeconomic factors, medical service background, and patient selection process for clinical trials.

RRs in phase III studies including third-generation cytotoxic agents seemed to be distributed randomly from one study to another, whereas patient survival might have been better in Japanese studies. The Japanese phase III trials were performed in academic institutes, including university-affiliated hospitals, cancer center hospitals, and central city general hospitals. Thus, the distribution of patients selected for Japanese phase III trials may be skewed, in that they were in good general condition, although established prognostic factors in patients with NSCLC were almost identical among Japanese and non-Japanese studies. In addition, better survival among Japanese patients may be attributable to true ethnic differences. One possibility is the relatively high frequency of non-squamous histology in Japanese studies, but the reason is largely unknown.

The severity and frequency of common toxicity were comparable in all these phase III studies with a few exceptions. The incidence of grade 3-4 neutropenia was only 5–6% in the carboplatin and paclitaxel arm of the INTACT2 study [12] and in the cisplatin and gemcitabine arm of the INTACT1 study [11], both of which were sponsored by one pharmaceutical company. Similarly, the incidence of neutropenia was lower in Greek studies [13–15] than in other studies. These differences in the incidence of toxicity may be associated with the frequency of monitoring, including patient hospital visits and blood cell count and chemistry evaluation.

Table 3 The combination of cisplatin and gemcitabine

| Characteristics | Japan [6] | Italy [21] | Spain [22] | EORTC [23] | EU [11] | ECOG [9] | EU + USA [24] |
|---|---|---|---|---|---|---|---|
| Chemotherapy dose | | | | | | | |
| CDDP ($mg/m^2$) | 80 (day 1) | 100 (day 2) | 100 (day 1) | 80 (day 1) | 80 (day 1) | 100 (day 1) | 100 (day 1) |
| GEM ($mg/m^2$) | 1000 (day 1, 8) | 1000 (day 1, 8, 15) | 1250 (day 1, 8) | 1250 (day 1, 8) | 1250 (day 1, 8) | 1000 (day 1, 8, 15) | 1000 (day 1, 8, 15) |
| Demographics (% not specified) | | | | | | | |
| No. of patients | 146 | 155 | 182 | 160 | 363 | 288 | 260 |
| Age (median) (range) | 61 (34–74) | 62 (28–76) | 59 (33–75) | 57 (28–75) | 61 (33–81) | 64 (32–87) | 62 (36–88) |
| Female | 33 | 37 | 12 | 29 | 28 | 37 | 30 |
| PS 0–1 | 100 | 93 | 85 | 89 | 90 | 95 | 80 |
| Stage IV | 81 | 79 | 77 | 79 | 69 | 86 | 67 |
| Non-squamous | 81 | 68 | 55 | 74 | 71 | NA | 70 |
| Treatment delivery and efficacy (% not specified) | | | | | | | |
| Cycles (median) | 3 | NA | 4 | 5 | 6 | 3 | 4 |
| Response rate (95% CI) | 30 (23–38) | 38 (30–46) | 42 (35–50) | 37 (29–45) | 47 (42–53) | 22 (17–27) | 30 (25–36) |
| MST (month) (95% CI (month)) | 14.8 (NA) | 8.6 (NA) | 9.3 (8.1–10.5) | 8.9 (7.8–10.5) | 10.9 (NA) | 8.1 (7.2–9.4) | 9.1 (8.3–10.6) |
| 1-year survival | 60 | 33 | 38 | 33 | 44 | 36 | 39 |
| Grade 3-4 toxicity (%) | | | | | | | |
| Neutropenia | 63 | 40 | 32 | 43 | 5 | 63 | 57 |
| Febrile neutropenia | 2 | 1 | 4 | 3 | NA | 4 | 5 |
| Thrombocytopenia | 35 | 64 | 19 | 36 | 6 | 50 | 50 |

Table 4 The combination of cisplatin and vinorelbine

| Characteristics | Japan [6] | Greece [15] | France [25] | EU [26] | SWOG [19] | USA [10] |
|---|---|---|---|---|---|---|
| Chemotherapy dose | | | | | | |
| CDDP ($mg/m^2$) | 80 (day 1) | 80 (day 8) | 100 (day 1) | 120 (day 1) | 100 (day 1) | 100 (day 1) |
| VNR ($mg/m^2$) | 25 (day 1, 8) | 30 (day 1, 8) | 30 (day 1, 8, 15, 22) | 30 (day 1, 8, 15, 22) | 25 (day 1, 8, 15, 22) | 25 (day 1, 8, 15, 22) |
| Demographics (% not specified) | | | | | | |
| No. of patients | 145 | 204 | 156 | 206 | 202 | 404 |
| Age (median) (range) | 61 (28–74) | 64 (46–75) | 57 (39–74) | 59 (NA) | 61 (32–83) | 61 (35–80) |
| Female | 30 | 25 | 21 | 12 | 33 | 25 |
| PS 0–1 | 100 | 90 | 92 | 80 | 100 | 96 |
| Stage IV | 83 | 64 | 86 | 59 | 89 | 67 |
| Non-squamous | 81 | 54 | 76 | 44 | NA | 65 |
| Treatment delivery and efficacy (% not specified) | | | | | | |
| Cycles (median) | 3 | 4 | 4 | 3 | NA | 4 |
| Response rate (95% CI) | 33 (25–41) | 39 (33–46) | 36 (28–43) | 28 (22–34) | 28 (22–34) | 25 (20–29) |
| MST (month) (95% CI (month)) | 11.4 (NA) | 9.7 (8.3–11.2) | 9.6 (8.1–12.2) | 9.3 (NA) | 8.1 (6.7–9.6) | 10.1 (9.2–11.3) |
| 1-year survival | 48 | 41 | 42 | 37 | 36 | 41 |
| Grade 3-4 toxicity (%) | | | | | | |
| Neutropenia | 88 | 37 | 83 | 79 | 76 | 79 |
| Febrile neutropenia | 18 | 11 | 22 | 4 | 1 | 5 |
| Thrombocytopenia | 1 | 6 | 3 | 3 | 4 | 4 |

Anticancer agents are considered to be sensitive to ethnic factors, because of a steep pharmacodynamic curve for both efficacy and safety, a narrow therapeutic dose range, non-linear pharmacokinetics, their metabolic enzymes with the potential for drug-drug interaction, and these enzymes with the potential for ethnically variable activity caused by genetic polymorphism. Thus, bridging studies using pharmacologic endpoints are extremely important to apply efficacy, safety, and dose data from one place to another [16]. These pharmacologic studies can be incorporated into phase I trials and, when it is necessary, phase II trials. Furthermore, the current study suggests that, once the pharmacological property and recommended dose of a new cytotoxic agent are established in one country, the outcome of randomized controlled trials developed in other countries can be extrapolated to the population.

We defined ethnic populations in the current study according to the country where the study was performed. However, patients enrolled into multicenter European and North American studies may include patients with a diverse ethnicity. It would be greatly interesting to see RR, MST and toxicity in subgroups of patients with different ethnicity in those trials, although there has been no such data published.

Randomization of patients in a trial guarantees the comparability between treatment arms within the trial, but not between treatment arms in different trials. Thus, it is impossible to compare the outcome of different trials exactly. Nevertheless, we frequently refer to the outcome of trials performed outside Japan and they furnish us with much information. To compensate this limitation, we tried to compare patient characteristics between trials, but other factors including the frequency of monitoring may also affect the outcome greatly. The number of combination regimens evaluated in this study is insufficient, but no large scale Japanese trials of other combination regimens have been available so far.

This study failed to demonstrate whether this approach to clinical trial analysis was really helpful. For future clinical trials, consistency in monitoring, as well as the use of the common toxicity and response criteria, is important to keep comparability between trials. A meta-analysis using individual patient data may be more useful than a subgroup analysis within a trial to compare the outcomes between ethnic subgroups with adequate statistical power.

A phase II study of gefitinib in patients with advanced NSCLC who had previously received one or two chemotherapy regimens was conducted in cooperation with 43 hospitals across Europe, Australia, South Africa, and Japan. The population was prospectively stratified into Japanese and non-Japanese patients to investigate whether there were any differences between the two patient populations with respect to efficacy [17]. This study clearly showed that a global study of NSCLC using the same protocol was completed, and this global strategy was an effective method to speed up the development of a new anticancer agent in Japan. In addition, the stratification by the county or ethnicity is important in a global study of an investigational new drug to investigate geographical differences in efficacy and toxicity.

In conclusion, the dose of anticancer agents, RR, survival and toxicity of lung cancer chemotherapy showed some differences among Japanese, European, and USA studies. However, extrapolation of clinical data in a country to another population and global clinical trials including many countries were considered possible with adequate dose adjustment based on dose finding studies using a carefully projected protocol.

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# CT-guided needle biopsy of lung lesions: A survey of severe complication based on 9783 biopsies in Japan

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**Abstract**

*Purpose:* The aim of our study was to update the rate of severe complications following CT-guided needle biopsy in Japan via a mailed survey.

*Materials and methods:* Postal questionnaires regarding CT-guided needle biopsy were sent out to multiple hospitals in Japan. The questions regarded: the total number and duration of CT-guided lung biopsies performed at each hospital, and the complication rates and numbers of pneumothorax, hemothorax, air embolism, tumor seeding, tension pneumothorax and other rare complications. Each severe complication was followed with additional questions.

*Results:* Data from 9783 biopsies was collected from 124 centers. Pneumothorax was the most common complication, and occurred in 2412 (35%) of 6881 cases. A total of 39 (35%) hospitals reported 74 (0.75%) cases with severe complications. There were six cases (0.061%) with air embolism, six cases (0.061%) with tumor seeding at the site of the biopsy route, 10 cases (0.10%) with tension pneumothorax, six cases (0.061%) with severe pulmonary hemorrhage or hemoptysis, nine cases (0.092%) with hemothorax, and 27 cases (0.26%) with others, including heart arrest, shock, and respiratory arrest. From a total of 62 patients with severe complications, 54 patients (0.55%) recovered without sequela, however one patient (0.01%) recovered with hemiplegia due to cerebral infarction, and the remaining seven patients (0.07%) died.

*Conclusions:* This is the first national study documenting severe complications with respect to CT-guided needle biopsy in Japan. The complication rate in Japan is comparable to internationally published figures. We believe this data will improve both clinicians as well as patients understanding of the risk versus benefit of CT-guided needle biopsy, resulting better decisions.



*Keywords:* CT-guided needle biopsy; Complication; Lung nodule

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## 1. Introduction

Transthoracic needle biopsy is a common procedure used mainly to elucidate the nature of pulmonary nodules [1,2]. CT has rapidly become the guidance modality of choice for performing transthoracic needle biopsy due to technical advances in CT and its better detection of pulmonary lesions, which sometimes cannot be identified on chest radiograph [3].

CT-guided needle biopsy is generally regarded as a safe procedure, although pneumothorax and other rare complications can sometimes occur [4]. There have been occasional reports of deaths due to severe complications, such as, air embolism following lung biopsy [5]. Fortunately, these complications are generally very rare; previously published data shows wide variations in complication rates, making them difficult to generalize [5–8].

The aim of our study was to update the rate of severe complications following CT-guided needle biopsy in Japan via a mailed survey.

## 2. Materials and methods

Postal questionnaires regarding CT-guided needle biopsy were sent out to named radiologists at 101 university hospitals and cancer centers in Japan in August 2001. The radiologists at these hospitals were asked to pass duplications of the questions to other associate hospitals. The questions required information regarding: the total number and duration of CT-guided lung biopsies performed at each hospital, and the complication rates, numbers of pneumothorax, hemothorax, air embolism, tumor seeding, tension pneumothorax, severe pulmonary hemorrhage or hemoptysis which was treated with drugs for hemostasis and other rare complications, and mortalities and morbidities after that.

We defined a case as having a severe complication when one of the following criteria was met: (1) the duration of hospital stay was prolonged due to the biopsy, (2) a special technique or treatment was required to treat the complication, (3) a special procedure was required for resuscitation, and (4) shock or pre-shock developed. Each severe complication was followed with additional questions, including diagnosis of the complication, the position of the pulmonary lesion, the distance of the pulmonary lesion from the peripheral pleura, whether the lesion was located near the hilum or large pulmonary vessel, whether there was any reasonable factor causing the complication such as cough during biopsy, biopsy technique (CT-fluoroscopy or Co-axial method), the number of biopsies for each case, type and size of the needle, and presence of significant sequela from the complication.

Furthermore, the questionnaire included the following enquiries: whether emergency medication was prepared for resuscitation in the operating room, whether the patient was treated by the intravenous route and monitors, such as automatic sphygmomanometer, pulse oximetry, and electrocardiography. Finally, availability of access to other departments in case of emergency was questioned. Postal replies of questionnaire had been received for a year, and these answers were analyzed.

## 3. Results

A total of 9783 biopsy data were collected from 124 centers. The average number of biopsies performed per center was 79 cases, and that per center per year was 21 cases. The number of institutions in which hyperbaric oxygen recompression can be performed was 41 of 114 (37%) hospitals. Patients were kept on peripheral intravenous drip infusion in 86 of 92 (93%) hospitals, automatic sphygmomanometer in 38 of 92 (41%) hospitals, pulse oximetry in 32 of 92 (35%) hospitals, and electrocardiography in 8 of 92 (9%) hospitals.

Pneumothorax was the most common complication, and occurred in 2412 (35%) of 6881 cases. The number of centers that reported severe complications was 39 (35%) of 114 centers. The total number of overall severe complications was 74 (0.75%) cases. Of these, details of the complications in 64 cases are described in Table 1. There were six cases (0.061%) with air embolism, six cases (0.061%) with tumor seeding at the site of the biopsy route, 10 cases (0.10%) with tension pneumothorax, six cases (0.061%) with severe pulmonary hemorrhage or hemoptysis, 10 cases (0.10%) with hemothorax, and 26 cases (0.26%) with others. The others included 14 cases of pneumothorax requiring temporal drainage of the pneumothorax or chest tube insertion, three cases of heart arrest, and so on. There was no report of coughing during needle placement into the thorax in any of the cases with air embolism. Two of six pulmonary lesions were complicated with air emboli located near the large pulmonary vessel, and one lesion contained a cavity (Table 2). Tumor seeding occurred in two cases following CT-guided biopsy performed

Table 1
Summary of 64 cases of severe complications

| Severe complications | No. |
|---|---|
| Pneumothorax requiring drainage of air | 14 |
| Tension pneumothorax | 10 |
| Hemothorax | 10 |
| Air embolism | 6 |
| Tumor seeding | 6 |
| Pulmonary hemorrhage of hemoptysis | 6 |
| Heart arrest | 3 |
| Respiratory arrest | 1 |
| Shock | 1 |
| Cyanosis | 1 |
| Cardiac tamponade | 1 |
| Pneumomediastinum | 1 |
| Mediastinal hematoma | 1 |
| Loss of consciousness | 1 |
| Severe pain of biopsied site | 1 |
| disseminated intravascular coagulation (DIC) | 1 |
| Total | 64 |

Table 2
Summary of cases of air embolism

| No. | Age | Sex | Size (mm) | Location (lobe) | Distance from pleura (mm) | Large vessel near the nodule | Cavity | CT-fluoloscopy | Co-axial method | No. of biopsy | Technique of biopsy | Size of the needle | Sequela |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 72 | F | 20 | Left lower | 40 | Yes | No | Yes | No | 2 | Core biopsy | 18G | Death |
| 2 | 59 | M | 10 | Left lower | 20 | No | No | NA[a] | Yes | 1 | Core biopsy | 18G | Totally improved |
| 3 | 57 | F | 7 | Right middle | 25 | No | No | Yes | No | 1 | Core biopsy | 18G | Totally improved |
| 4 | 74 | M | 20 | Right upper | 25 | Yes | No | Yes | No | 2 | Core biopsy | 20G | Partially improved |
| 5 | 57 | M | 12 | Right lower | 3 | No | No | No | Yes | 1 | Core biopsy | 20G | Totally improved |
| 6 | 75 | M | 25 | Right lower | 18 | No | Yes | No | No | 1 | Core biopsy | 18G | Totally improved |

[a] NA, information was not available.

by the Co-axial method (Table 3). In one of these two cases, the tip of the outer cannula was placed within the chest wall, so that seeding obviously occurred by direct contact of the inner needle with the biopsy route.

From a total of 62 cases with severe complications, 54 cases (0.55%) were recovered without sequela, and one case (0.01%) recovered but with hemiplegia due to cerebral infarction. Unfortunately, four (0.04%) of the remaining seven cases died just after the CT-guided biopsy procedure; these consisted of one case of air embolism, one case of DIC, and two cases of heart arrest. Three cases (0.03%) of the remaining seven cases died several years later due to tumor seeding. Four cases complicated with air embolism, three of which were treated with hyperbaric oxygen recompression, were recovered without sequela out of a total of six cases. In 23 (50%) of 46 centers, an emergency team was able to attend when a severe complication occurred.

## 4. Discussion

Recently, many small pulmonary lesions, which cannot be detected on chest radiograph, have been easily visualized by CT examination in daily clinical work. These lesions are usually followed with CT, or in some cases these are biopsies using CT-guided technique. CT-guided needle biopsy is a widely accepted technique and is one of the principal methods for evaluating a pulmonary lesion [9]. Although it is not rare to have minor complications due to CT-guided needle biopsy, such as, a small amount of pneumothorax and pulmonary hemorrhage, these complications improve without any treatment [5]. On the other hand, it is well known that potentially life-threatening complications such as air embolism and tumor seeding can occur. Fortunately, the frequency of these complications is considered very rare [5]. However, the number of published reports has shown that the incidence of air embolism has been increasing over the last several years. Only seven cases with air embolism were documented in the 20 years before 1995 [10–16], whereas six cases have already been published in the last 10 years [17–22].

This is the first national research study demonstrating the incidence rate of severe complications with respect to CT-guided needle biopsy based on a large number of biopsy cases using a multi-center survey.

The most common complication of transthoracic percutaneous needle biopsy is pneumothorax, with a frequency rate of 0–61%, whereas the incidence of pneumothorax requiring chest tube drainage ranges from 1.6% to 17% [23]. In the present study, the rate of pneumothorax was 35.1%, which is considered comparable to the previous studies.

Sinner's review of the literature determined that there were two cases suspected of air embolism in 2726 patients [5]. He estimated that the relative risk of air embolism per patient was about 0.07%. In the present study of 9783 biopsies, air embolism occurred in six patients, resulting in an incidence