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Local Control of Regional and Metastatic Lesions and Indication for Systemic Chemotherapy in Patients with Non-Small Cell Lung Cancer

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ABSTRACT

Systemic chemotherapy is the mainstay of treatment in patients with advanced non-small cell lung cancer. Local control of regional and metastatic lesions may be needed before systemic therapy can be started in patients with pleural effusions or bone or brain metastases. The indication for systemic chemotherapy depends on the symptoms and performance status of the patient. In addition, a risk assessment considering complications such as hemodynamic and respiratory compromise by effusions, pathological bone fractures, and neurologic deterioration caused by brain metastases is critical in selecting which patients should receive first-line systemic chemotherapy before local therapy, although predictive factors for these complications have not yet been established. Chemotherapy has been considered to have only a limited role in the treatment of patients with pleural effusions and brain and bone metastases, but recently developed anticancer agents have shown substantial antitumor effects in these types of patients with a good general condition. *The Oncologist* 2008:13(suppl 1):21–27

INTRODUCTION

The majority of patients with non-small cell lung cancer (NSCLC) develop distant metastases either by the time of the initial diagnosis or during recurrence following surgery for the primary lesion. While systemic chemotherapy is the mainstay of treatment in patients with advanced NSCLC, local control of regional and metastatic lesions may be needed before systemic therapy can be used in patients with pleural effusions, bone metastases, or brain metastases. The general rule about whether local control should precede systemic chemotherapy varies according to the performance status (PS) of a patient and the responsiveness of the tumor to chemotherapy. If possible, systemic chemotherapy should be employed early in patients with malignant lymphoma and germ-cell tumors, as they are highly responsive and can be cured even at an advanced stage. It is unlikely that small-cell lung cancer can be cured, but because it responds well to chemotherapy, chemotherapeutic agents are frequently given prior to local therapy. In patients with advanced NSCLC, however, local therapy is often required before chemotherapy is administered because of the limited efficacy of chemotherapy in these patients.

PLEURAL EFFUSIONS

Malignant pleural effusions are a common clinical problem in patients with neoplastic disease, and may be the first presenting sign in as many as 10% of patients. Indeed, approximately 15% of lung cancer patients present with malignant pleural effusions at diagnosis [1]. In fact, lung cancer is the most common cause of malignant pleural effusions,

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accounting for 17%–56% of cases [2]. Dyspnea is the most common symptom in patients with malignant effusions, occurring in more than half of cases, followed by cough and chest pain, although 5%–25% of patients have no respiratory complaints [3].

PS is significantly associated with survival in patients with pleural effusions [4]. Pleural effusions have been treated with the aim of palliation because NSCLC patients with pleural effusions are advanced stage by definition; massive effusions can cause hemodynamic and respiratory compromise, and the development of a symptomatic pleural effusion can drastically alter the quality of life and survival of patients [2]. Recently, however, as a result of the availability of ultrasound, computed tomography (CT), and positron emission tomography scans, NSCLC patients with small, asymptomatic pleural effusions can now be identified, and the treatment approach can be reconsidered in the setting of systemic disease control because relatively effective chemotherapy regimens have been developed.

It should be noted that pleural effusions can affect drug pharmacokinetics: methotrexate administered i.v. to patients with massive effusions is slowly released from third-space fluid, resulting in prolongation of the terminal half-life of the drug in the plasma, and potentially also increasing its toxicity [5, 6]. Similarly, levels of 5-fluorouracil decline rapidly in the plasma, but persist for longer in the effusion [7]. The pharmacokinetics of other drugs in patients with effusions are poorly studied, but drugs may accumulate in effusions and only slowly be redistributed throughout the body [8].

Patients with a small pleural effusion causing no symptoms can be treated with primary systemic chemotherapy, although there is a risk that the effusion will become symptomatic and require therapy. Patients with effusion-related dyspnea and those with a massive pleural effusion should be treated with a therapeutic thoracentesis; a large-volume thoracentesis allows rapid relief of symptoms in many patients. If systemic disease progression is a significant concern, an initial thoracentesis may create a window of opportunity in which to gain control over symptoms before starting chemotherapy. For patients whose effusions recur rapidly, more aggressive interventions may be required to achieve durable palliation, including chest tube drainage followed by chemical pleurodesis, and thoracoscopy with talc poudrage [8]. If patients gain durable palliation and are restored to a good PS by these treatments, then systemic chemotherapy is indicated. If not, their condition is suggestive of terminal-stage disease with a very short life expectancy.

Patients with NSCLC and pleural effusions are commonly included in chemotherapy clinical trials while they retain a good PS. Although the control of effusions by systemic chemotherapy has rarely been described, the efficacy of chemotherapy in treating effusions is considered to be comparable to the systemic response to chemotherapy. A retrospective study of 34 NSCLC patients with malignant pleural effusions treated with cisplatin, ifosfamide, and irinotecan showed that effusions disappeared for >4 weeks in 13 (38%) patients, while a partial response in measurable primary or metastatic lesions was obtained in 25 (66%) patients [9]. Active mutations of epidermal growth factor receptor (EGFR) have been detected in samples of pleural effusion fluid, and in patients with NSCLC they were associated with a clinical response to gefitinib, an EGFR tyrosine kinase inhibitor [10]. These results suggest that, in the near future, investigation of pleural effusion fluid could be important in selecting a chemotherapy regimen in patients with advanced NSCLC.

Brain Metastases

Lung cancer is the most common primary source of brain metastases, which develop in 10%–64% of lung cancer patients during the clinical course of the disease [11]. Even among newly diagnosed, asymptomatic patients with potentially operable NSCLC, routine brain scans identify brain metastases in 3%–10% of patients [12]. It is believed that the incidence of brain metastases is increasing as a result of an aging population, better control of extracerebral disease by more active systemic therapy, and better detection of small metastases following the development of imaging modalities such as magnetic resonance imaging (MRI).

Two thirds of cancer patients found to have brain metastases at autopsy had experienced neurologic symptoms resulting from the metastases, with only 10% of patients diagnosed by CT or MRI between 1973 and 1993 being asymptomatic [13]. Symptoms include headache, focal weakness, nausea, vomiting, and altered mental status. Seizures occur in about 20% of patients with brain metastases. When lung cancer patients are routinely screened, only 10% present to the physician with symptoms of brain metastases [12]. Thus, although the exact percentage is unknown, there are many patients with NSCLC who have brain metastases but no neurologic symptoms. The prognosis for patients with brain metastases is influenced largely by PS, age, and control of the primary and extracranial tumors. Whole brain radiotherapy (WBRT), with or without stereotactic irradiation, has been the treatment of choice for most patients with brain metastases, with a median survival time of 3–6 months after radiotherapy. This relatively short survival is related to progressive systemic disease rather than the brain metastases [11]. Therefore, systemic chemotherapy can be administered in many patients with brain metastases and is in fact important for their survival.

Chemotherapy has not been thought to have a major role in the treatment of patients with brain metastases because of a poor PS in many cases and the prevailing belief that the blood–brain barrier (BBB) may play a role in limiting delivery of chemotherapeutic agents to the central nervous system. However, the accumulation of contrast medium during CT or MRI assessments and the development of edema surrounding metastatic lesions suggest that tumor-induced vessels do not possess normal anatomical and physiological properties, and the BBB at the site of established brain metastases may be partly disrupted [14]. While one study demonstrated that the concentration of cisplatin in the brain metastases of patients who received the agent before surgery did not differ from that found in extracranial metastases [15], another study found that paclitaxel concentration in brain metastases was in the therapeutic range, while in brain tissue the concentration was below the limit of detection [16]. This observation is supported by objective response rates of brain metastases to systemic chemotherapy of 27%–50% in previously untreated patients with NSCLC, which are comparable to systemic response rates (Table 1) [17–23]. Gefitinib has also been shown to be effective against brain metastases arising from NSCLC; objective responses were obtained in 13 of 25 case reports of gefitinib use in such patients [24]. Thus, systemic chemotherapy is an important treatment option for NSCLC patients with brain metastases, as long as a good PS is maintained without neurologic symptoms.

The advantages of administering chemotherapy before radiotherapy can be summarized as follows: (a) it is useful to judge the tumor's response to chemotherapy; (b) radiotherapy decreases blood supply to the tumor and thus may hamper the ability of chemotherapeutic agents to reach the metastases; and (c) chemotherapy delivered before radiotherapy may be less toxic to the brain than chemotherapy after radiotherapy, because radiotherapy may open the BBB and allow the entry of potentially neurotoxic agents. Evidence for this is available for methotrexate treatment, and may also apply to other agents [25]. A randomized phase III trial of cisplatin plus vinorelbine followed by WBRT (arm A; $n = 86$) versus the same chemotherapy with early concurrent WBRT (arm B; $n = 85$) in NSCLC patients with brain metastases showed that the respective intracranial response rates evaluated after two cycles of chemotherapy were 27% and 33%, and that the overall response rates were 21% and 20%. The median survival time was 5.5 months in arm A and 4.8 months in arm B ($p = .83$). There was no difference between the arms in terms of hematologic and neurologic toxicities. These results suggest that chemotherapy is effective against brain metastases arising from NSCLC, and that the timing (early or delayed) of WBRT does not influence the survival of these patients [21].

Bone Metastases

Bone metastases are common in patients with lung cancer, with an incidence of 30%–55% at autopsy. These metastases are usually osteolytic, and are distributed mainly in

Table 1. Chemotherapy in previously untreated non-small cell lung cancer patients with brain metastases

Study	Chemotherapy regimen	*n* of patients	Response rate (%)		Median survival time (months)
			Intracranial	Systemic	
Minotti et al. (1998) [17]	CDDP + TNP	23	35	30	4.8
Crinò et al. (1999) [18]	CDDP + IFM + MMC	120	39	23	NA
	CDDP + GEM	123	41	37	NA
Franciosi et al. (1999) [19]	CDDP + ETP	43	30	75	7.4
Fujita et al. (2000) [20]	CDDP + IFM + CPT	30	50	62	12.6
Robinet et al. (2001) [21]	CDDP + VNR	86	27	35	5.5
Bernardo et al. (2002) [22]	CBDCA + VNR + GEM	20	45	45	7.6
Cortes et al. (2003) [23]	CDDP + PTX + VNR or GEM	25	38	50	4.9

Abbreviations: CBDCA, carboplatin; CDDP, cisplatin; CPT, irinotecan; ETP, etoposide; GEM, gemcitabine; IFM, ifosfamide; MMC, mitomycin-C; NA, not available; PTX, paclitaxel; TNP, teniposide; VNR, vinorelbine.

the spine, pelvis, ribs, and extremities. The most common symptom of bone metastases is pain, which is either diffuse or localized. It is characteristically described as dull and constant in presentation, worsening at night. The pain gradually increases in intensity, and can be exacerbated by certain movements or positions, such as standing, walking, or sitting [26]. However, up to 25% of patients with bone metastases are free of pain, and patients with multiple bone metastases typically report pain in only a few sites. The factors that convert a painless lesion to a painful one are unknown [27]. As bone destruction progresses, mechanical weakness and loss of structural integrity lead to pathological fracture; spinal instability, defined as mechanical instability in the spine related to extensive bone destruction [28]; cord compression, and hypercalcemia [26, 29]. The prognosis for patients with bone metastases varies among the different tumor types. The median survival time from diagnosis of bone metastases in patients with prostate cancer or breast cancer is measurable in years, whereas for lung cancer it is only 6–7 months [29]. The second most important prognostic factor in patients with bone metastases is PS; the median survival time for patients with a Karnofsky PS score of <50, 50–70, or 80–100 who received radiotherapy to the metastatic site was 2–3 months, 5 months, and 12 months, respectively [30, 31].

Bone destruction and its complications severely limit the activity and mobility of patients. For patients with a high risk for these complications, radiotherapy is the treatment of choice and orthopedic interventions may be necessary in some cases [26, 29].

Pathologic fractures occur in 8%–30% of all cancer patients, with the ribs, vertebrae, and long bones being the most frequent fracture sites [26, 29]. A long-bone fracture, especially when located at the proximal part of the femur, has a detrimental effect on the quality of life of patients with advanced cancer. Important factors in predicting an impending fracture of the long bones are pain that is exacerbated by movement and radiographic findings such as a predominantly osteolytic appearance, a large lesion, and axial cortical involvement [32, 33].

Spinal instability is the cause of back pain in 10% of patients with advanced cancer [26]. It can cause unbearable pain that is mechanical in origin, and frequently the patient is only comfortable when lying still [26]. Neither radiation therapy nor chemotherapy, even if successful in controlling the tumor, will alleviate the pain. As in the treatment of pathological fractures of the long bones, stabilization of the vertebral segments is required for pain relief [28]. However, major surgery is associated with significant morbidity and mortality, and good results can be obtained only in carefully selected patients. Percutaneous vertebroplasty provides rapid and effective relief from the pain associated with spinal instability.

Spinal cord compression occurs in 2%–5% of cancer patients [34]. The incidence varies with the type of cancer, and is 2.6% for NSCLC [35]. The cumulative incidence for all cancers decreases with age: it is 4.4% for patients aged 40–50 years, 3.9% for patients aged 50–60 years, 2.9% for patients aged 60–70 years, 1.7% for patients aged 70–80 years, and 0.5% for those aged >80 years [34]. About 60%–80% of spinal cord compressions occur in the thoracic region, 15%–30% in the lumbar region, and 10% in the cervical region. Multiple compression sites occur in approximately 7%–14% of cases [26, 34]. Early diagnosis and treatment are important for successful rehabilitation, but 48%–96% of patients present with motor weakness, bladder dysfunction, and inability to walk. In 83%–96% of patients, the first symptom is pain at the affected site, which may have been present from as little as 1 day to as long as 2 years, with a median duration of 8 weeks. It is generally exacerbated by coughing, sneezing, and straining, and typically increases in intensity over several weeks. Thus, the development of back pain in a cancer patient is a warning sign for possible spinal cord compression [26, 34].

Asymptomatic patients with bone metastases are potentially candidates for initial systemic chemotherapy, unless they show no risk factors for structural complications in radiographic assessments. These patients have been included in clinical trials of systemic chemotherapy; however, only limited information is available on the efficacy of chemotherapy for bone metastases, mainly because it is difficult to assess response to treatment in the bone, and bone metastases are defined as nontarget lesions in the Response Evaluation Criteria in Solid Tumors [36]. In patients with breast cancer, objective response rates of osteolytic lesions to standard chemotherapy regimens vary in the range of 20%–60% [37]. There are currently no reports on the objective response of bone metastases to chemotherapy in patients with NSCLC, but pain relief has been observed in 30%–61% of NSCLC patients receiving cisplatin-based chemotherapy, gemcitabine, or gefitinib [38-40].

Bisphosphonates, pyrophosphate analogues with a phosphorus–carbon–phosphorus (P–C–P)-containing central structure that promotes binding to the mineralized bone matrix, provide an additional treatment strategy for metastatic bone disease. Approximately 25%–40% of i.v. administered bisphosphonates are excreted by the kidney, and the remainder binds avidly to exposed bone mineral around resorbing osteoclasts, leading to inhibition of bone resorption and apoptosis of osteoclasts [26]. In addition to clinical use for hypercalcemia of malignancy, bisphos-

phonates are a routine treatment to prevent skeletal-related events (SREs) in patients with metastatic breast cancer and multiple myeloma. A recent meta-analysis evaluating randomized trials in these patients that lasted for 6 months or longer showed that bisphosphonates led to a significantly lower risk, versus placebo, for vertebral fractures (odds ratio [OR], 0.69; 95% confidence interval [CI], 0.57–0.84), nonvertebral fractures (OR, 0.65; CI, 0.64–0.99), radiotherapy (OR, 0.67; CI, 0.57–0.79), and hypercalcemia (OR, 0.54; CI, 0.36–0.81). In contrast, trials of <6 months' duration did not show any significant results for any skeletal morbidity outcome [41]. In patients with NSCLC, however, the role of bisphosphonates in the treatment of bone metastases has been less investigated. A recent phase III trial of zoledronic acid, a new generation bisphosphonate that has 100-1,000 times the potency of pamidronate in vitro, showed that 4 mg zoledronic acid led to a significantly lower annual incidence of SREs (1.74 per year versus 2.71 per year; $p = .012$) and longer median time to first SRE (7.8 months versus 5.1 months; $p = .009$) compared with placebo in 773 patients with lung cancer and other solid tumors [42, 43]. There are no criteria regarding the indication and duration of bisphosphonate therapy in patients with NSCLC. Evidence of bone destruction on plain radiographs, which is suggestive of receiving a benefit of bisphosphonates in patients with breast cancer [44], also may be an important factor in patients with NSCLC.

The presence or absence of bone pain should not be a factor in initiating bisphosphonates in patients with breast cancer [44], but no reports are available on this issue in patients with NSCLC. Because a relatively long duration of treatment (>6 months) is required for patients to get a benefit from bisphosphonates, patient prognosis is considered another factor to determine the indication of this type of agent [26].

Treatment Algorithm

Pleural effusions, brain metastases, bone metastases, and their associated morbidities give rise to a vexing clinical problem in patients with advanced NSCLC. Approaches to treating these patients are illustrated in Figure 1. The use of systemic chemotherapy depends on the symptoms and PS of the patients. In addition, a risk assessment looking at complications is critical in selecting which patients should receive first-line systemic chemotherapy, although factors predictive of these complications have not yet been established. Chemotherapy has previously been considered to have only a limited role in the treatment of patients with pleural effusions and brain and bone metastases, but recently developed anticancer agents have been shown to have substantial antitumor effects in patients with a good general condition.

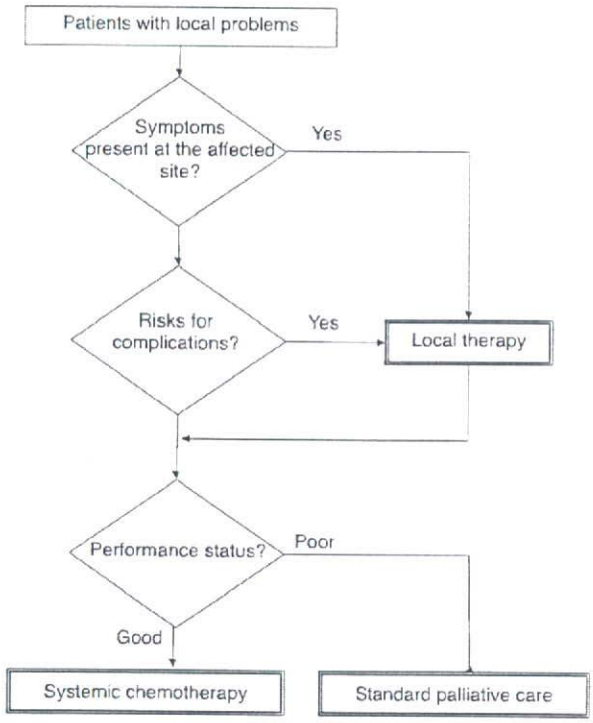

Figure 1. Treatment approaches for patients who have advanced non-small cell lung cancer with local problems.

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Original article

Relation between elective nodal failure and irradiated volume in non-small-cell lung cancer (NSCLC) treated with radiotherapy using conventional fields and doses

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ABSTRACT

Introduction: The role of elective nodal irradiation of non-small-cell lung cancer (NSCLC) patients treated with radiotherapy remains unclear. We investigated the significance of treating clinically uninvolved lymph nodes by retrospectively analyzing the relationship between loco-regional failure and the irradiated volume.

Methods: Between 1998 and 2003, patients with IA–IIIB NSCLC were treated with radiotherapy. The eligibility criteria for this study were an irradiation dose of 60 Gy or more and a clinical response better than stable disease. Typical radiotherapy consisted of 40 Gy/20 fr to the tumor volumes (clinical target volume of the primary tumor [CTVp], of the metastatic lymph nodes [CTVn], and of the subclinical nodal region [CTVs]), followed by off-cord boost to CTVp+n to a total dose 60–68 Gy/30–34 fr. The relationship between the sites of recurrence and irradiated volumes was analyzed.

Results: A total of 127 patients fulfilled the eligibility criteria. Their median overall and progression-free survival times were 23.5 (range, 4.2–109.7) and 9.0 months (2.2–109.7), respectively. At a median follow-up time of 50.5 months (range, 14.2–83.0) for the surviving patients, the first treatment failure was observed in 95 patients (loco-regional; 41, distant; 42, both; 12). Among the patients with loco-regional failure, in-field recurrence occurred in 38 patients, and four CTVs recurrences associated with CTVp+n failure were observed. No isolated recurrence in CTVs was observed.

Conclusions: In-field loco-regional failure, as well as distant metastasis, was a major type of failure, and there was no isolated elective nodal failure. Radiation volume adequacy did not seem to affect elective nodal failure.

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Radiation therapy is an integral component of the multi-modal treatment of non-small-cell lung cancer (NSCLC). Recent phase III studies have demonstrated that concomitant chemoradiotherapy improves survival, and this has resulted in the general acceptance of concurrent chemoradiotherapy as one of the standard treatments for locally advanced NSCLC [1]. Despite the improved survival, however, most patients die from their disease as a result of local or distant failure.

Local failure remains a major challenge when treating NSCLC with radiotherapy. A number of studies of dose escalation to the gross tumor volume (GTV) have been conducted as a means of improving local control [2–5]. The conventional radiation fields for NSCLC typically encompass the entire mediastinum and ipsilateral hilum (elective nodal region) to deliver a dose of 40 Gy, even without evidence of disease in these areas, followed by a 20 Gy boost to the GTV. However, the conventional treatment has added considerable morbidity and can limit the dose escalation. In phase I–II dose escalation studies, there is a trend toward omitting the practice of elective nodal irradiation (ENI) after their experiences with toxicity, which is not based on direct evidence [2–5]. According to those studies, omitting ENI has not sacrificed treatment outcomes so far. They also analyzed patterns of recurrence in relation to irradiated volume in a dose escalation setting [6].

By contrast, the current literature provides limited information regarding patterns of failure when conventional fields and doses are used [7,8]. Since it is important to know whether loco-regional failure is within or outside the irradiation field, we retrospectively analyzed patterns of failure after radiation therapy for NSCLC, especially in regard to the relationship between local failure and irradiated volume.

Methods and materials

Patients

Between January 1998 and March 2003, 263 patients with newly diagnosed NSCLC were treated with thoracic radiation therapy,

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with or without chemotherapy, at the National Cancer Center Hospital. All tumors were cytologically or histologically confirmed NSCLC. Patients' disease was staged by the tumor-node-metastasis (TNM) staging system (UICC, version 6, 2002). The diagnostic workup included a bone scan, brain scan by computed tomography (CT) or magnetic resonance imaging, CT scan of the chest, and CT or ultrasound imaging of the abdomen. The criteria for inclusion in this study were irradiation with a dose of 60 Gy or more as a part of the initial treatment and a clinical response better than stable disease. After excluding patients with metastatic disease, whose primary tumor was located in the apex of the lung (superior sulcus), and whose post-treatment evaluation was inadequate, the remaining 127 patients served as the subjects of the analysis.

Details of treatment

Radiotherapy

Gross tumor volume (GTV) was defined as the demonstrable extent of the primary tumor and the metastatic lymph nodes, GTVp and GTVn, respectively. GTVn was defined as abnormally enlarged regional lymph nodes measuring over 1.0 cm along their short axis. Clinical target volume (CTV) consisted of the adjacent mediastinum and ipsilateral hilum (CTV of the subclinical nodal region, CTVs) as well as CTVp and CTVn which were assumed to be equal to GTVp and GTVn, respectively. A planning target volume (PTV) margin of 1–1.5 cm was drawn around each CTV.

External-beam radiotherapy with a 6, 10, or 15 MV photon beam was delivered using a linear accelerator. A majority of the patients were treated with anteroposterior opposing fields encompassing CTV to a dose of 40 Gy/20 fractions (2 Gy per fraction, 5 days per week), followed by an off-cord boost to the GTV by oblique opposing fields, to a total dose of 60–68 Gy/30–34 fractions. No attempt was made to encompass the supraclavicular areas in most patients; the supraclavicular areas were treated only electively. Initially, treatment planning was performed by using an X-ray simulator for the anteroposterior fields and a CT-port for the oblique opposing fields, but after the end of 1999, most treatment planning, especially to define the off-cord boost, was performed using a CT-based planning system (FOCUS, Computed Medical Systems).

The dose to the spinal cord was limited to 45–50 Gy. The size of the treatment fields was adjusted so that it did not exceed half of the hemithorax before introducing CT-based planning system, or so that the volume of normal lung tissue receiving a dose over 20 Gy would be less than 40%.

Chemotherapy

Systemic chemotherapy was used in 87 patients (68.5%), and the majority of the patients received platinum-based chemotherapy sequentially or concurrently with the radiation therapy. One of the representative regimens was 2–3 cycles of cisplatin 80 mg/sqm on day 1 and vinorelbine 25 mg/sqm on days 1 and 8 (or vindesine 3 mg/sqm on days 1, 8, and 15) in 21–28 days. The second most common regimen was cisplatin 80 mg/sqm on day 1, vindesine 3 mg/sqm on days 1 and 8, and mitomycin C 8 mg/sqm on day 1, in 21–28 days. The other regimens are summarized in Table 1.

Evaluation

Patients were followed at 4- to 6-week intervals for 6 months after treatment and at 3- to 6-month intervals thereafter. Chest X-ray and laboratory workups were performed at each post-treatment visit. Unless there were changes in the chest X-ray or in symptoms, a CT scan was performed about 2–3 months after the treatment for the assessment of the treatment response, and every 6–12 months thereafter. Follow-up information was obtained from the medical charts and death certificates.

Table 1
Baseline patient characteristics.

Characteristics	Patients	(%)
Median age (yr)	65 (36–83)	
Gender		
Male	106	83
Female	21	17
Performance status (WHO)		
0	12	9
1	109	86
2	6	5
Stage		
I (A/B)	5(1/4)	4
II (A/B)	12(3/9)	9
III (A/B)	110(59/51)	87
Histology		
Adenocarcinoma	64	50
Squamous cell carcinoma	39	31
Large cell carcinoma	4	3
NSCLC (not otherwise specified)	20	16
Chemotherapy (concurrent/sequential)	87(63/24)	69
Chemotherapy regimens		
Cisplatin + vindesine or vinorelbine	48	55
Carboplatin + paclitaxel	12	14
MVP (cisplatin + vindesine + mitomycin)	12	14
Nedaplatin or nedaplatin + paclitaxel	11	13
Others	4	5

When evaluating overall survival, an event was defined as death from any cause. When evaluating progression-free survival, an event was defined as documented tumor progression (loco-regional or distant) or death from any cause. Local or loco-regional failure was judged to have occurred if there was radiographic evidence of progressive disease. Absence of progression of residual disease for more than 6 months following treatment was considered evidence of loco-regional control. A recurrence in supraclavicular nodes was considered regional failure, not an elective nodal failure, because the supraclavicular regions are not routinely included within the radiation fields in our practice. Treatment failure was not always confirmed histologically. Elective nodal failure (ENF) was defined as recurrence in CTVs without evidence of local failure, as the first event or even after distant metastasis.

The adequacy of field borders was assessed in terms of CTVs coverage and PTV margin in patients with loco-regional failure. The failure patterns were analyzed to distinguish in-field recurrence from out-of-field recurrence; "in-field" included CTVs as well as CTVp and CTVn.

The Kaplan–Meier method was used from the start of the treatment to calculate the overall survival and progression-free survival of all the 127 patients.

Results

A total of 127 patients, median age 65 years (range, 36–83), met the criteria for evaluation in this study. The majority of patients had stage IIIA ($n = 59$) or IIIB ($n = 51$) disease. Other baseline characteristics of the patients and details of their treatment are summarized in Table 1.

At a median follow-up time of 50.5 months (range, 14.2–83.0) of the surviving patients, 95 had experienced treatment failure. Median survival time was 23.5 months (range, 4.2–109.7), and median time to progression was 9.0 months (range, 2.2–109.7). The 2-year cumulative survival rate and 2-year progression-free survival rate were 51.4% and 27.6%, respectively. The survival

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curves are shown in Fig. 1. Patients with early progressions were excluded because of the criteria for inclusion in this study: a clinical response better than stable disease.

Eighty-seven (69%) patients received chemotherapy concomitantly or sequentially with the radiotherapy. The overall survival time of the patients who received chemotherapy was 21.7 months (range, 7.6–33.9), as opposed to 19.1 months (range, 6.8–32.7) among those who did not receive chemotherapy, and the difference was not statistically significant ($p = 0.10$). There were no statistically significant differences in disease-free survival nor loco-regional control according to whether the patients had received chemotherapy. Concurrent use of chemoradiotherapy did not affect survival among the 87 patients who received chemotherapy (data not shown).

There were 53 patients with a first loco-regional failure, alone ($n = 41$) or with distant metastasis ($n = 12$), and the majority of the failures were in-field ($n = 38$, 72%). Nine (21%) patients had out-of-field recurrences in the form of supraclavicular node metastasis ($n = 5$) or pleural metastasis ($n = 4$), with or without local recurrence. There were no isolated ENFs (Table 2).

Four patients (7%) experienced nodal failure in CTVs simultaneously with local or distant failure. Three of them had received a prophylactic dose of 40 Gy to the CTVs, and the other had inadequate margin of the CTVs field. Other characteristics of these patients are shown in Table 3. There were no "marginal only" failures among in-field failures; all the failures at the field borders were associated with out-of-field failures.

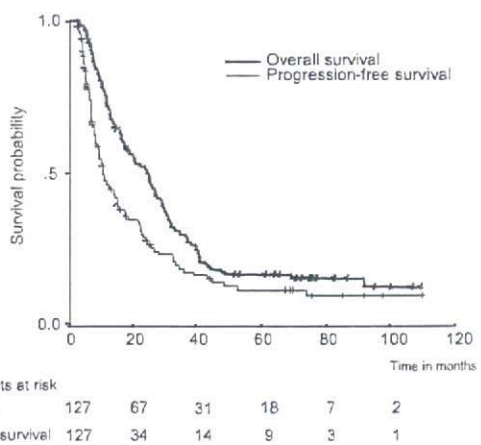

Fig. 1. Overall and progression-free survival curves of all the 127 patients. Patients with early progressions were excluded because of the criteria for inclusion in this study: a clinical response better than stable disease.

Table 2
Details of all the first failures.

Types of event	Patients	%
Loco-regional alone	41	43%
In-field		
CTVpn	30	
CTVpn + CTVs[a]	2	
In-field + out-of-field		
CTVpn + pleural effusion	2	
CTVpn + supraclavicular nodes	2	
Out-of-field		
Supraclavicular nodes	3	
Pleural effusion[b]	2	
Loco-regional + distant	12	13%
In-field + out-of-field		
CTVpn + CTVs	2	
Distant alone	42	44%
All events	95	

[a] One also had concurrent failure in the contralateral hilum.
[b] One also had concurrent supraclavicular recurrence.

Conventional X-ray simulation was performed in 8 (6%) patients, while 70 (55%) had CT-based simulation and remaining 49 (39%) had both (initially with X-ray simulation, followed by CT-based simulation for off-cord boost). A majority ($n = 122$, 96%) of the patients were treated with anteroposterior opposing fields as elective nodal irradiation, followed by oblique opposing fields to the total dose.

ENI was incomplete ($n = 12$) or not performed ($n = 6$) in 18 of the 53 patients with loco-regional failure because of diminished pulmonary function or deteriorated performance status. All the incomplete ENIs were due to insufficient CTVs coverage. In 12 of the 18 patients, the failure was in the tumor volume, in 3 patients it was in the pleura, and in 2 patients it was in the supraclavicular nodes. Only 1 patient had recurrence in both the tumor volume and the uninvolved nodal area.

Discussion

In this series of NSCLC cases treated with conventional fields and doses, the loco-regional failures after radiotherapy mainly occurred in the tumor volumes, and there were no isolated ENFs.

There are several possible reasons for these results. First, micrometastasis in the CTVs may have been controlled by prophylactic delivery of 40 Gy to the region, and depending on the location of the primary tumor, the sites of occult metastasis may often have received additional unintentional radiation doses. Kepka et al. reported an isolated ENF rate of 9% in 185 patients treated with the ENI using 3-dimensional conformal radiotherapy (3D-CRT). Their analysis showed that the ENF occurred more frequently in the regions that received under 40 Gy than in the regions that received higher doses (69% vs. 31%, respectively, $p = 0.04$) [7]. However, despite the same ENF rate of 9% in 1705 patients in the four trials conducted by the Radiation Therapy Oncology Group (RTOG), a retrospective evaluation of in-field progression revealed that neither in-field progression nor survival was affected by the adequacy of ENI [8]. Field adequacy did not have any negative impact on regional control in our series either (Tables 3).

Second, the amount of micrometastasis in unenlarged mediastinal regional nodes may have been small enough to be controlled by chemotherapy, which has been shown to have activity that reduces the incidence of distant micrometastasis in advanced NSCLC. However, the degree of systemic and local efficacy of chemotherapy did not reach statistical significance in our series, probably because of the small number of patients and their heterogeneity (data not shown).

Third, since the failure sites in the majority of patients were distant, they would have died of their disease before the ENF became apparent. As a result, the loco-regional failure rates may have been lower than their true values because we did not investigate regional sites once a patient developed distant metastasis.

The therapeutic significance of treating subclinical nodal regions during and after surgery for NSCLC has been questioned. Some studies have established the presence of considerable microscopic nodal disease in clinically uninvolved lymph nodes [9,10], but the role of mediastinal lymphadenectomy remains controversial and has been limited to the precise staging of the disease [11–13]. A study by Izbicki et al. which compared systemic mediastinal lymphadenectomy with mediastinal lymph node sampling showed that radical systemic mediastinal lymphadenectomy had no effect on the disease-free or overall survival of patients with limited nodal involvement [13,14]. The role of adjuvant radiotherapy after complete resection also remains unclear [15–17]. A sys-

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Table 3
Patients with CTVs failure.

	Patient #1	Patient #2	Patient #3	Patient #4
Age (yr)/Sex	45/Female	74/Female	61/Male	78/Male
Reason for inoperability	Unresectable	Unresectable	Decreased pulmonary function	Unresectable, age
Stage	IIIA	IIIA	IIB	IIIB
Primary location	Left lower lobe	Right upper lobe	Right lower lobe	Left upper lobe
Histology	Adenocarcinoma	Adenocarcinoma	Squamous cell carcinoma	Adenocarcinoma
Chemotherapy	Yes	Yes	No	No
Response	Partial response	Partial response	Partial response	Partial response
Site of first failure	Distant and loco-regional	Distant and loco-regional	Loco-regional	Loco-regional
Field border adequacy	Yes	Yes	No	Yes
Dose to CTVs failure	40	40	0	40
Death	No	No	Yes	No

temic review and meta-analysis [18] showed that postoperative radiotherapy was detrimental to patients with early NSCLC, although there may have been some efficacy in patients with N2 tumors. These arguments also raise questions about the clear benefit of ENI in regard to survival.

In-field loco-regional failure was a major site of failure in the current study: all the recurrences in the CTVs were associated with failure in the gross tumor volume. Thus, more intensive treatment strategies are needed to enhance loco-regional control without sacrificing safety. One possible strategy is to reduce the ENI field in regard to the patients' risk factors while escalating the total dose. Such an attempt has already been made in regard to surgery: Asamura et al. retrospectively reviewed the prevalence of lymph node metastasis with respect to the location of the primary tumor or other characteristics to decide on the optimal lobe-specific extent of systematic lymph node dissection for NSCLC [19,20]. By using such predictors, including the location of the primary tumor, histology, or nodal stage [21–24], it is possible to identify the nodal areas at risk and to optimize the extent of ENI in radiation therapy as well. On the other hand, more precise diagnosis by novel technology, such as positron emission tomography [25], may enable the omission of ENI and avoid unnecessary irradiation to areas at low risk for subclinical disease.

In terms of the technical feasibility of dose escalation, Grills et al. found that intensity-modulated radiation therapy without ENI for NSCLC increased the deliverable mean target dose in node-positive patients by 25–30% over 3D-CRT and by 130–140% over traditional ENI [26].

Because omitting ENI is likely to leave microscopic disease untreated, there is concern that it may result in increased failure in these areas. However, the preliminary results of dose escalation trials have shown that isolated ENF outside the irradiated volume occurred in fewer than 6% of the cases and that omission of ENI did not seem to sacrifice outcome [2–5,27]. There is insufficient evidence to support the use of ENI for any patient with localized NSCLC (Stages I–III), irrespective of whether chemotherapy is administered [28]. There has been only one randomized trial that compared high-dose thoracic radiotherapy without ENI and standard dose radiotherapy with ENI, and it showed a survival benefit of high-dose thoracic radiotherapy without ENI [29]. One possible explanation for this finding is that incidental doses to elective nodal areas may contribute to the eradication of the subclinical disease. The pattern of ENF according to nodal regions was described by Rosenzweig et al., who implemented the use of involved-field radiation therapy with dose escalation in 524 patients [6]. Since the majority of the 42 ENFs that were observed occurred in the areas that received less than 45 Gy, the incidental doses to elective nodal areas may have been substantial despite the attempt not to treat these regions in their study. In addition, Zhao et al. reported that involved-field radiation therapy with a dose escalated to 70 Gy delivered a considerable dose to CTVs, and when the primary tumor was large or centrally located, the percentages of CTVs in the lower paratracheal region, subcarinal region and ipsilateral hilar region receiving over 40 Gy were 33%, 39%, and 98%, respectively [30].

Because of the retrospective nature of our study, no conclusions about the value of ENI for NSCLC can be drawn. However, the finding that in-field loco-regional failure, as well as distant metastasis, was a major type of failure with the standard field and dose of thoracic radiotherapy confirmed the need for more intensive treatment.

Further investigation to verify the true significance of ENI or to identify best candidates for ENI is necessary before it is abandoned in the context of dose escalation.

Conclusion

The loco-regional failures after radiotherapy in this series of NSCLC cases treated with conventional fields and doses mainly occurred in the tumor volumes, and there were no isolated ENFs. The results confirmed the need for more intense treatment to improve local control.

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Prospective Study of the Accuracy of *EGFR* Mutational Analysis by High-Resolution Melting Analysis in Small Samples Obtained from Patients with Non–Small Cell Lung Cancer

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Abstract

Purpose: Epidermal growth factor receptor (*EGFR*) mutations, especially in-frame deletions in exon 19 (DEL) and a point mutation in exon 21 (L858R), predict gefitinib sensitivity in patients with non–small cell lung cancer (NSCLC). In this study, we verified the accuracy of *EGFR* mutation analysis in small samples by high-resolution melting analysis (HRMA), which is a rapid method using PCR amplification with a dye to analyze the melting curves in NSCLC.

Experimental Design: We designed a prospective study to compare the sensitivity and specificity of HRMA and DNA sequencing with laser capture microdissection. Eligible patients with lung lesions were screened by bronchoscopy or percutaneous needle biopsy to histologically confirm the diagnosis, followed by surgical resection of the NSCLC. Small diagnostic specimens were analyzed for *EGFR* mutations by HRMA, and the surgically resected specimens were examined for mutations by HRMA and DNA sequencing.

Results: The analyses for *EGFR* mutations were conducted in 52 eligible cases of the 92 enrolled patients. *EGFR* mutations were detected in 18 (34.6%) patients. The results of HRMA from surgically resected specimens as well as DNA sequencing revealed 100% sensitivity and specificity. On the other hand, the sensitivity and specificity of HRMA from the small diagnostic specimens were 83.3% and 100%, respectively.

Conclusions: In this study, we showed that HRMA is a highly accurate method for detecting DEL and L858R mutations in patients with NSCLC, although it is necessary to consider the identification of patients with a false-negative result when the analysis is conducted using small samples.

Somatic mutations in the kinase domain of the epidermal growth factor receptor (*EGFR*) have been reported in patients with non–small cell lung cancer (NSCLC; refs. 1–3). Although many types of *EGFR* mutations have been identified, they seem to be concentrated in exons 18 to 21 of *EGFR*; ~85% to 90% of *EGFR*-mutant patients have mutations in two hotspots: a short in-frame deletion in exon 19 (DEL) and a point mutation at codon 858 in exon 21 (L858R; ref. 4). Several studies have revealed that *EGFR* mutations are strongly associated with the tumor response and clinical outcome in patients with NSCLC receiving treatment with EGFR tyrosine kinase inhibitors, such as gefitinib (Iressa, AstraZeneca; refs. 5–7). The mutational status of *EGFR*, especially the presence/absence of DEL and L858R, is a strong predictor of the sensitivity to EGFR tyrosine kinase inhibitor, and the detection of *EGFR* mutations is useful for decision-making by both patients and physicians (4, 8). Recently, a laboratory test for *EGFR* mutations has become clinically available for guiding treatment decisions.

Until now, screening for these mutations has most commonly been conducted using DNA sequencing methods. In our previous study, we used methanol-fixed, paraffin-embedded surgical specimens and performed direct sequencing and pyrosequencing with laser capture microdissection (LCM) to ensure high-quality genetic analysis of archived tissues (5, 9). However, these approaches are not useful in clinical practice for two reasons. First, although the sequencing methods require a high ratio of tumor-to-normal tissue DNA for optimal results, the diagnostic specimens obtained from cases of advanced NSCLC may contain only a small amount of tumor cells and

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are highly contaminated with normal cells. Secondly, *EGFR* mutation analysis based on DNA sequencing requires special instruments and is also time-consuming and expensive. Therefore, some simple and highly sensitive nonsequencing methods to detect *EGFR* mutations have been reported (10–22). However, the accuracy of these methods for clinical use have not been assessed in prospective studies.

High-resolution melting analysis (HRMA) using the LCGreen I (Idaho Technology) dye was introduced as an easy, quick, and inexpensive method for the screening of mutations (23), and we established and validated the HRMA method to detect DEL and L858R mutations in cases of NSCLC (9, 10). Our cell line study revealed that DEL and L858R mutations could be detected using HRMA in the presence of 10% and 0.1% of mutant cells, respectively (10). We also showed that the two major mutations could be identified by HRMA retrospectively using DNA extracted from archived Papanicolaou-stained cytologic slides with 88% sensitivity and 100% specificity (9). Furthermore, it was shown that among patients treated with gefitinib, the response rate (78% versus 8%), time-to-progression (median, 9.2 versus 1.6 months), and overall survival (median, 21.7 versus 8.7 months) were significantly better in patients with *EGFR* mutations than with wild-type *EGFR* ($P < 0.001$), as detected by HRMA (9). These results suggest that this easy, quick, and inexpensive method which was done using diagnostic small samples of advanced NSCLC tumors is one of the most useful and precise methods to detect *EGFR* mutations in clinical practice.

In this study, we designed a prospective study to detect two major *EGFR* mutations by HRMA using small diagnostic cytologic or biopsy specimens and surgically resected specimens, and the results were compared with the results of DNA sequencing methods combined with LCM, which we consider as the "gold standard" for such detection, applied to methanol-fixed, paraffin-embedded surgically resected specimens. We evaluated the diagnostic sensitivity, specificity, predictive values, and accuracy of the detection of *EGFR* mutations using HRMA and revealed that this method is feasible for clinical use to detect *EGFR* mutations in small samples obtained from patients with NSCLC.

Patients and Methods

Patients and materials. Patients with lung lesions, which were suspected clinically to be operable NSCLC, were enrolled in this prospective study. The patients were scheduled for bronchoscopy or percutaneous needle biopsy to establish the histologic diagnosis, and informed consent was obtained from each of the patients prior to these diagnostic procedures. Thereafter, the patients diagnosed with NSCLC underwent lung surgery at our hospital. In this study, mutational analysis of *EGFR* was done by HRMA or DNA sequencing methods combined with LCM in all the patients in which both the preoperatively obtained diagnostic specimens and the resected specimens were histologically confirmed by a certified pathologist to contain malignant cells.

Based on a protocol approved by the Institutional Review Board of the National Cancer Center, we did mutational analyses of *EGFR* to detect DEL and L858R in the eligible patients. The Papanicolaou-stained cytologic slides ($n = 35$), formalin-fixed, paraffin-embedded transbronchial or percutaneous needle biopsy specimens ($n = 34$), and methanol-fixed, paraffin-embedded surgically resected specimens subjected to LCM using a PixCell II LCM system (Arcturus Engineering, Inc.; $n = 52$) were collected prospectively. DNA was extracted using the QIAamp DNA Micro Kit (Qiagen), as described in our previous report (10).

HRMA. PCR was done to amplify exons 19 or 21 of *EGFR* using LCGreen I (Idaho Technology) on a LightCycler (Roche Diagnostics) and primers designed as previously described (10). If the first PCR products were not available for the mutational analyses of the melting curves, we did a second PCR using the same primers. These PCR products were denatured at 95°C for 10 min and cooled to 40°C to promote the formation of heteroduplexes. The LightCycler capillary was transferred to an HR-1 (Idaho Technology), an HRMA instrument, and heated at a transition rate of 0.3°C/s. Data were acquired and analyzed using the accompanying software (Idaho Technology). After normalization and temperature-adjustment steps, melting curve shapes from 78.5°C to 85.5°C were compared between the tumor samples and control samples. Human Genomic DNA (Roche Diagnostics) was used as the negative control sample with wild-type *EGFR*. Samples revealing skewed or left-shifted curves as compared with the control samples were judged to have mutations without positive controls (9, 10). All analyses were done in a blinded fashion by two researchers (T. Fukui and T. Takano). After independent evaluation by the two researchers, the final judgment was arrived at by consensus after joint viewing of the melting curves from both.

DNA sequencing methods with LCM. In our previous study, we did a direct sequencing or pyrosequencing of *EGFR* in patients with recurrent NSCLC after primary surgery (5). Based on the results of our previous study, we consider direct sequencing with LCM for the detection of DEL and pyrosequencing with LCM for the detection of L858R as the gold standard in relation to *EGFR* mutational analysis. DNA was extracted from methanol-fixed, paraffin-embedded surgical specimens by LCM, according to a previously described method (24). Direct sequencing of the PCR products for DEL was done using ABI PRISM3700 and 3100 DNA sequencers (Applied Biosystems). Pyrosequencing to analyze L858R was done using Pyrosequencing PSQ 96MA (Pyrosequencing; refs. 5, 25). The *EGFR* mutational analysis using DNA sequencing methods was done in a blinded fashion by a researcher (H. Sakamoto) according to a previously described method (5), and then compared with the corresponding results obtained using HRMA.

Statistical analysis. The primary end point of this study was the sensitivity and specificity of the results obtained using HRMA as compared with those of the results obtained using DNA sequencing with LCM. The sample size was calculated using a statistical power level of 0.80 and two-sided α level of 0.1 on the basis of an estimated sensitivity of at least 0.80 and an expected value of 0.95 for HRMA, a minimum of 20 patients with *EGFR*-mutated tumors were required. Because the percentage of NSCLC patients with *EGFR* mutations was expected to be 40% in this study population composed of only Japanese, approximately 50 patients with NSCLC were needed. Therefore, considering a specificity of at least 0.80 and the expected value of 0.95 for HRMA, 30 patients with wild-type tumors showed a statistical power level of 0.90 using a two-sided α level of 0.1.

The associations between mutational status and patient characteristics were assessed by a χ^2 test using the SPSS statistical package (SPSS version 11.0 for Windows; SPCC, Inc.).

Results

Patient characteristics. From December 2005 to December 2006, 92 patients with clinically suspected operable NSCLC were enrolled in this study. The following diagnostic procedures were done preoperatively in 90 patients: bronchoscopy ($n = 57$), percutaneous needle biopsy ($n = 27$), or bronchoscopy followed by percutaneous needle biopsy ($n = 6$). The patient characteristics are shown in Table 1. All the patients were Japanese. Among the patients, a definitive diagnosis was established in 85 patients by bronchoscopy in 43 of 59 patients

Table 1. Patient characteristics

(A) Characteristics of all the patients enrolled in this study (n = 92)

	All (n = 92)	BF (n = 64)	PNB (n = 34)*
Age, year, median (range)	64 (34-84)	64 (38-84)	62 (41-79)
Gender (male/female)	58/34	41/23	23/11
Smoking history (N/F/C)	29/30/33	23/19/22	7/14/13
Tumor size, mm, average (range)	27.2 (10.2-73.4)	28.3 (13.8-56.6)	24.5 (10.2-73.4)
Accuracy of the diagnostic procedure (%)	66/85 (77.6)	43/59 (72.9)	25/31 (80.6)
Accuracy of the cytologic slides (%)	54/85 (63.5)	31/59 (52.5)	23/30 (76.7)
Accuracy of the biopsy specimens (%)	42/62 (67.7)	35/54 (64.8)	7/9 (77.8)

(B) Characteristics of the patients who underwent analysis of the EGFR mutations in this study (n = 52)

	All (n = 52)	BF (n = 38)	PNB (n = 17)†
Age, year, median (range)	64.5 (34-84)	64.5 (34-84)	64 (47-78)
Gender (male/female)	36/16	25/13	14/3
Smoking history (N/F/C)	16/17/19	15/11/12	1/7/9
Tumor size, mm, average (range)	27.0 (11.0-56.6)	28.3 (20.6-56.6)	24.1 (11.0-48.8)
Postoperative diagnosis (Ad/Sq/LCNEC)	45/5/2	34/4/0	12/3/2
Pathologic stage (IA/B, IIA/B, IIIA/B)	19/13, 3/5, 9/2	15/8, 3/2, 8/2	7/5, 0/2, 3/0

NOTE: Never smokers were defined as patients who had never smoked, former smokers were defined as patients who had stopped smoking at least 1 y before the diagnosis, and current smokers were defined as patients who were still smoking at the time of the diagnosis.
Abbreviations: BF, bronchoscopy; PNB, percutaneous needle biopsy; N, never smoker; F, former smoker; C, current smoker; Ad, adenocarcinoma; Sq, squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma.
*Including six patients in whom bronchoscopy was done followed by percutaneous needle biopsy.
†Including three in whom bronchoscopy was done followed by percutaneous needle biopsy.

(72.9%) and by percutaneous needle biopsy in 25 of 31 patients (80.6%); in 18 of the 85 (21.2%) patients, the histologic diagnosis could not be established preoperatively by bronchoscopy and/or percutaneous needle biopsy, the patients underwent lung surgery for suspicious malignant lung lesion, and examination of the resected specimens revealed the diagnosis of primary NSCLC in 17 and malignant lymphoma in 1 of the 18 patients. Among the 76 patients diagnosed to have primary NSCLC, 73 consented to undergo lung surgery. Finally, the analysis for *EGFR* mutations was done on 52 patients with a definitive histologic diagnosis of primary NSCLC, established both by examination of the preoperative diagnostic specimens and of the corresponding resected specimens (Fig. 1).

Mutational analyses. We analyzed 35 cytologic samples and 34 biopsy specimens obtained from 52 patients by HRMA, and

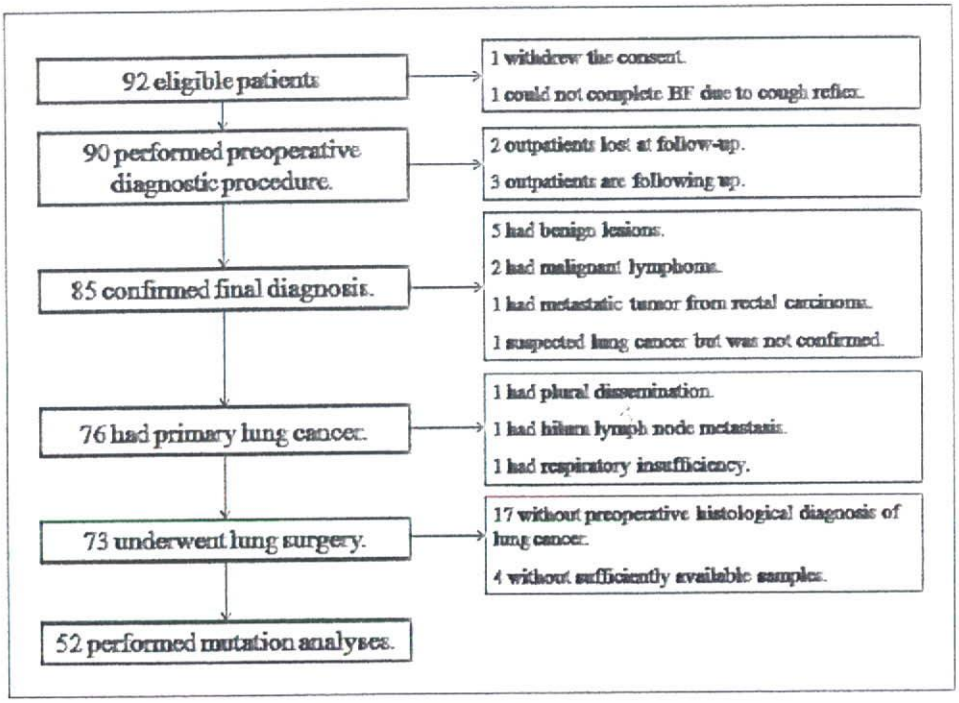

Fig. 1. Flowchart of the analyses conducted in 92 enrolled patients with lung tumors in this study.

Table 2. EGFR mutation status among the patient subgroups

	n	*EGFR* mutations*				*P*
		DEL	L858R	Total	%	
Total	52	5	13	18	34.6	—
Gender						
Women	16	2	9	11	68.8	0.001
Men	36	3	4	7	19.4	
Smoking history						
Never	16	3	8	11	68.8	0.001†
Former	17	2	4	6	35.3	
Current	19	0	1	1	5.3	
Histology						
Ad	44	5	13	18	100	0.025‡
Sq	6	0	0	0	0	
LCNEC	2	0	0	0	0	

Abbreviations: DEL, deletional mutations in exon 19; L858R, a point mutation at codon 858 in exon 21; Ad, adenocarcinoma; Sq, squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma.
*The EGFR mutations were analyzed by DNA sequencing with LCM.
†Comparison between never smokers and others.
‡Comparison between adenocarcinoma and others.

did both HRMA and DNA sequencing with LCM in the 52 resected specimens corresponding to the 52 patients. Among the 52 surgically resected specimens analyzed by DNA sequencing with LCM, there were 18 (34.6%) samples with *EGFR* mutations, 5 with DEL mutations, and 13 with L858R mutations. As shown in Table 2, the *EGFR* mutations were detected more frequently in women, never-smokers, and patients with a histologic diagnosis of adenocarcinoma. All results from HRMA done in a blinded fashion by two researchers (T. Fukui and T. Takano) were consistent.

HRMA could be conducted using small diagnostic samples from all 52 patients, although the analysis needed to be conducted using the second PCR product in 15 cases. In the analysis of exon 19, 5 samples revealed different curves from the control and 47 samples revealed almost the same curves as the control; therefore, we judged that the five former patients had DEL mutations (Fig. 2A). In the analysis of exon 21, 10 samples revealed a left-shift from the control and 42 samples revealed almost the same curves as the control; therefore, we judged that the 10 former patients had L858R mutations

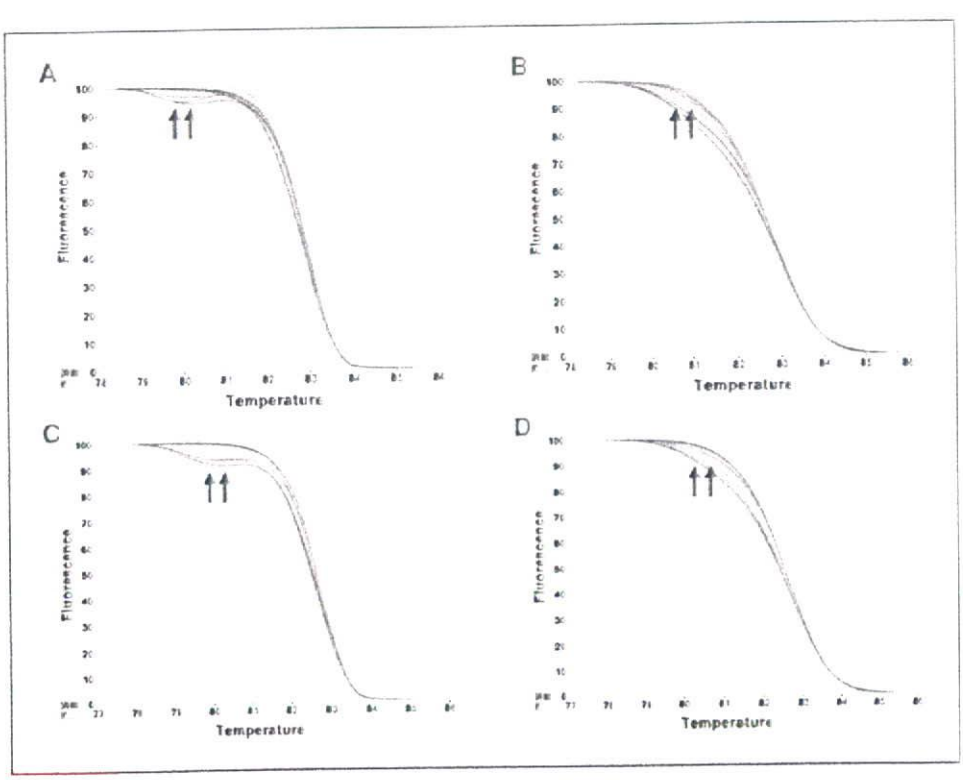

Fig. 2. Adjusted melting curves obtained by HRMA of the samples in this study to detect *EGFR* mutations (1), in-frame deletions in exon 19 (*A*, small samples; *C*, resected specimens) and a point mutation in exon 21 (*B*, small samples; *D*, resected specimens). Each sample that revealed a skewed or left-shifted curve from those of the control sample was judged to have a mutation.

Table 3. Results of the EGFR mutation analyses in patients with *EGFR* mutation-positive tumors

No. of patients	Small samples	Surgically resected specimens	
	HRMA	HRMA	Sequence with LCM
13	DEL	DEL	DEL1*
26	DEL	DEL	DEL1*
32	DEL	DEL	DEL2†
40	DEL	DEL	DEL2†
47	DEL	DEL	DEL1*
5	L858R‡	L858R	L858R
6	Wild-type	L858R	L858R
12	L858R	L858R	L858R
18	L858R	L858R	L858R
21	L858R	L858R	L858R
23	L858R‡	L858R	L858R
25	Wild-type	L858R	L858R
27	L858R‡	L858R	L858R
28	L858R	L858R	L858R
31	Wild-type‡	L858R	L858R
41	L858R‡	L858R	L858R
53	L858R	L858R	L858R
54	L858R‡	L858R	L858R

Abbreviations: DEL, deletional mutations in exon 19; L858R, a point mutation at codon 858 in exon 21.
*DEL1: del E746-A750 (del 2235-2249).
†DEL2: del E746-A750 (del 2236-2250).
‡The analyses by HRMA were done using second PCR products.

(Fig. 2B). All the 52 surgically resected specimens analyzed by DNA sequencing with LCM could also be analyzed by HRMA, although the analysis needed to be conducted using the second PCR product in two cases. DEL mutations were detected in 5 patients (Fig. 2C) and L858R mutations in 13 patients (Fig. 2D) among the 52 patients. Of the 52 specimens, both cytologic slides and biopsy specimens were analyzed in 17 cases. Discrepant results were obtained by HRMA in one of the cases, with L858R mutation being detected in the cytologic slides but not in the biopsy specimens. We included this patient in the population with L858R mutations.

The results of HRMA were consistent with the results of DNA sequencing with LCM in all the surgically resected specimens analyzed by the two methods. On the other hand, HRMA using small diagnostic specimens revealed the wild-type curve in three cases, although analysis of the corresponding surgically resected specimens analyzed by pyrosequencing with LCM revealed the L858R mutation (Table 3). Thus, the results for these samples obtained by HRMA were considered as false-negative results. Neither method of analysis yielded any false-positive cases. The results of the *EGFR* mutational analysis by HRMA compared with DNA sequencing with LCM using surgically resected specimens were shown in Table 4. The sensitivity, specificity, and accuracy of HRMA using small diagnostic specimens were 83.3%, 100%, and 94.2%, respectively. Using surgically resected specimens, those of HRMA were all 100%.

Discussion

In this prospective study, we showed the high accuracy of the HRMA method for detecting two major *EGFR* mutations, DEL and L858R in patients with NSCLC. The accuracy of HRMA was clearly equal to that of DNA sequencing with LCM for the detection of mutations in surgically resected specimens. On the other hand, the sensitivity and specificity of HRMA were 83.3% (90% confidence interval: 68.9-97.7%) and 100%, respectively, when the small diagnostic samples were analyzed. Although the sensitivity of HRMA which was estimated to be at least 0.80 did not reach statistical significance, we consider HRMA as one of the available methods for the detection of *EGFR* mutations in clinical practice because the specificity, which is important for clinical decision-making, of HRMA was 100% and the *EGFR* mutation rate was less than the expected 40% to secure enough statistical power in this study.

Recently, many researchers reported establishing simple and highly sensitive nonsequencing methods for detecting *EGFR* mutations using small tumor samples (11-22), and the results of several mutation analyses were correlated with the clinical outcome of EGFR tyrosine kinase inhibitor treatment (17-19). Using serial dilution studies, some researchers have reported methods that are able to detect mutations in samples containing ~0.1% to 10% mutated DNA (13, 14, 16-18, 20-22), as opposed to direct DNA sequencing which requires the presence of at least 10% to 30% of mutated DNA in the samples (18, 20). Additionally, several novel methods offered higher sensitivity and specificity than DNA sequencing to identify the mutations in clinical samples. But almost none of the methods were validated for diagnostic accuracy in a prospective study, and we therefore consider these methods to still be unsuitable for routine clinical examination. Although these nonsequencing methods were not mutually compared, based on our previous results of retrospectively verifying the accuracy of HRMA (9, 10), we thought to develop in this prospective study an easy, quick (PCR for ~1 hour and HRMA for 2 to 3 minutes), and inexpensive (at a running cost per sample of approximately $7.50, which consisted of $5.50 for the DNA extract and less than $2.00 for PCR using LCGreen I dye) method that might be useful in clinical practice with a great advantage over DNA sequencing, which requires the

Table 4. Comparison of the sensitivity, specificity, predictive values, and accuracy between HRMA and DNA sequencing with LCM (n = 52)

	HRMA using small samples	HRMA using surgically resected specimens
True-positive	15	18
True-negative	34	34
False-positive	0	0
False-negative	3	0
Sensitivity	83.3 (68.9-97.8)	100
Specificity	100	100
NPV	91.9 (84.5-99.3)	100
PPV	100	100
Accuracy	94.2 (88.9-99.5)	100

NOTE: The results of these analyses were compared with those of DNA sequencing with LCM (used as the gold standard in this study). Data are presented as % or % (90% confidence interval). True-positive is defined as the correct detection of DEL in exon 19 or L858R in exon 21.
Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

Table 5. Results of HRMA using cytologic slides or biopsy specimens

	Cytologic slides (n = 35)		Biopsy specimens (n = 34)	
	First PCR	Second PCR	First PCR	Second PCR
Successfully analyzed	29 (83.0%)	35 (100%)	5 (15.0%)	34 (100%)
True-positive	7	11	1	10
True-negative	19	21	4	22
True-negative	0	0	0	0
False-positive	3	3	0	2
Sensitivity	70.0% (7/10)	78.6% (11/14)	100% (1/1)	83.3% (10/12)
Specificity	100% (19/19)	100% (21/21)	100% (4/4)	100% (22/22)
NPV	100% (7/7)	100% (11/11)	100% (1/1)	100% (10/10)
PPV	86.4% (19/22)	87.5% (21/24)	100% (4/4)	91.2% (22/24)
Accuracy	89.7% (26/29)	91.4% (32/35)	100% (5/5)	94.1% (32/34)

NOTE: The results of these analyses were compared with those of DNA sequencing with LCM (used as the gold standard in this study). True-positive is defined as the correct detection of DEL in exon 19 or L858R in exon 21.
Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

extraction of high-quality DNA from an adequate amount of pure tumor cells, takes a long time, and is expensive.

In this study, the three patients with L858R detected by DNA pyrosequencing with LCM using the surgically resected specimens were labeled as having the wild-type *EGFR* in the analyses conducted using the small diagnostic samples. With regard to these false-negative results, the following three points need to be discussed: first, our previous study, conducted using human lung cancer cell lines, showed that HRMA can detect the mutations, even when samples contain only a small proportion (DEL, 10%; L858R, 0.1%) of mutant cells (10). In this study, the sensitivity of HRMA was also considered to be sufficiently high for the detection of *EGFR* mutations, especially L858R, even when the analysis was conducted using small samples after evaluation by a clinical pathologist to determine if they contained benign or malignant cells. Thus, we assume a higher accuracy of HRMA when using small samples in clinical practice. Although it still needs to be comparatively analyzed with the previously reported non-sequencing methods, HRMA can be considered as one of the sensitive methods available for the detection to *EGFR* mutations in clinical practice.

Second, high-quality DNA should be preserved in clinical samples to obtain the best results. There always remains the risk of an indeterminate or false-negative result because the DNA might have degenerated during sampling or during the preservation of clinical samples. In a comparison between the cytologic slides and biopsy specimens, better results were obtained from analyses of the first PCR products using the cytologic slides rather than the results obtained using the biopsy specimens, regardless of the amount of tumor cells examined (Table 5). This could probably be explained by the differences in the method of sample fixation between the two types of specimens. It has been suggested by a previous report that DNA is preserved better in the methanol-fixed samples than in the formalin-fixed specimens (26). Therefore, if we used methanol for specimen fixation of biopsy specimens, the results of HRMA using the first PCR products from small biopsy samples might improve. Hereafter, we propose to perform mutation analyses using methanol-fixed specimens, if possible.

Finally, we need to consider the possibility of intratumoral heterogeneity, and small diagnostic samples and surgically resected specimens may each represent overlapping but different populations of these tumor cells. A lack of association in the immunohistochemical expression profile between lung biopsy specimens and the corresponding resected tumor specimens has been reported (27). Furthermore, intratumoral heterogeneity was shown not only in terms of microheterogeneity of the tumor cell phenotype (28), but in terms of genetic heterogeneity in cancer (29, 30). In particular, the intratumoral genetic heterogeneity of *EGFR* mutations may explain the variable clinical response of NSCLC to gefitinib. It is also possible that the small diagnostic samples contain only wild-type cells, even if the tumor, overall, shows mutations, because the small samples yield only small part of the tumor. It is always necessary to consider the possibility of a false-negative result of mutational analyses conducted using the small samples.

In the current prospective study, we showed the feasibility and high accuracy of using HRMA for detecting two major *EGFR* mutations, DEL and L858R, in patients with NSCLC. Although HRMA showed high accuracy, the possibility of indeterminate or false-negative results, and because of the sensitivity of this method, the quality of DNA preservation in the clinical samples or intratumoral genetic heterogeneity, must be borne in mind to a certain extent when this analysis is conducted using small diagnostic samples. Therefore, HRMA should not be used to exclude patients from EGFR tyrosine kinase inhibitor treatment on the basis of the negative results only. Based on the results of this prospective study, we suggest that this method is very useful for clinical decision-making, especially in patients with a positive result.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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特集　気管食道領域の診断機器の進歩

総　説

診断機器の現状と将来の展望　気道領域

金子昌弘，土田敬明

国立がんセンター中央病院内視鏡部

要旨　呼吸器は気管から呼吸細気管支に至る管腔臓器の部分と，肺胞を中心にした実質臓器の部分からなっている。

気管から肺門部までの気道の診断は，ほとんどが電子気管支鏡による肉眼的な観察であるが，自家蛍光（AFI），狭帯域観察（NBI）や，超音波（EBUS）による診断も一部では行われている。将来的には拡大観察や，光コヒーレント断層（OCT）での観察などにより，内視鏡的に病理学的な診断が行えるようになることが期待されている。

肺門より末梢の気道の診断に以前は気管支造影が用いられていたが，被検者の負担も多く近年は行われていない。代わって高分解能CT画像からの気管支の3次元再構成や極細径気管支鏡の開発が行われており，今後の普及が期待されている。

末梢肺野病変の画像診断には高分解能CTやFDG-PETが用いられ，確定診断には経気管支鏡的生検が行われることが多い。生検器具の誘導に一般にはX線透視が行われているが，最近ではCTあるいは超音波ガイド下での生検も行われ，診断率の向上に役立っている。将来的には画像診断と内視鏡の誘導技術の更なる融合がはかられ，微小な異常部位から的確に標本の採取が可能なシステムの構築が望まれている。

キーワード：電子気管支鏡，自家蛍光，気管支超音波，バーチャル気管支鏡，CTガイド気管支鏡

I. はじめに

気道領域の中でわれわれが取り扱うのは，気管から肺胞に至る部分で，この部分は気管から呼吸細気管支に至る気道部分と肺胞を中心にした実質臓器の部分からなっており，前者はさらにⅢ次気管支程度までの肺門部分と，それより末梢の末梢気道部分に分けることができる。

一方，診断機器の面から見ると，内腔を直接観察する内視鏡と，放射線を中心とした画像診断の機器があり，前述の解剖学的な部位によりこれらが使い分けられ，また場合によっては両者が協力して診断が行われている。

本稿では肺がんの診断を中心に，これらの現状と将来の展望について考察する。

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II. 肺門部の診断機器

一般にⅡ次気管支の内腔の太さは5 mm程度なので，この付近までは通常の軟性気管支鏡による肉眼での観察や病変部位からの直視下の生検による診断が行われている。

気管支鏡は1970年代になり軟性気管支鏡（気管