【表3】術前放射線化学療法

Source	stage	n	chemotherapy	RT	response rate	resection rate	MST (surviv
CALGB [23]	ⅢAN2	41	FVP×2	30	51%	61%	16mo.
SWOG [24, 34]	ⅢAN2 ⅢB	75 51	PE×2	45	69% 45%	76% 63%	13mo. 17mo.
Rice [25]	ⅢAN2 ⅢB	42	PE×1	27	57%	79%	21mo.
Choi [26]	ⅢAN2	42	PVF×2	42	73%	93%	28mo. 37%：5y
Eberhardt [27]	ⅢAN2 ⅢB	94	PE×4	45		53%	31%：4y 26%：4y
Friedel [28]	ⅢA, ⅢB	93	PV	36 (seq)	69%	64%	24%：5y
Cyjon [29]	ⅢA, ⅢB	57	P-oral etopo.	45 (dailyP)	73% 69%	53%	16mo.
De Candis [30]	ⅢA, ⅢB	43	CBDCA＋Pac	50	52%	29%	15mo.
DeCamp [31]	ⅢA, ⅢB	105	P＋Pac P＋Pac	30 (pre) 30 (post)	NA	79%	27mo.

【表4】新規抗癌剤による術前治療

author	treatment	stage	n	CR＋PR (%)	resection rate	MST (m
Voltolini	PG	ⅢAN2 (media)	36	70%	50%	8
Yang	PG	Ⅲ	52	63%	69%	19
Cappuzzo	PG	ⅢAN2	129	62%	29%	19
Cigolari	PVn	ⅢB	30	60%	37%	25
Betticher [32]	PDoc	ⅢAN2 (media)	90	66%	83%	33

という良好な結果が報告された[18]．さらに，照射量も55～65Gyまで照射した報告もみられる[19, 20]．本邦においても，JCOGでsuperior sulcus tumorに対する2相試験が行われ，米国の報告と同等の結果が得られている[21]．

予後因子としては完全切除ができたことと縦隔リンパ節転移のなかったことがあげられており，切除を前提とする場合には治癒切除が可能で縦隔リンパ節転移がない症例を選択するべきであろう．

2．T4およびN2肺癌

T4肺癌は縦隔の重要臓器浸潤を伴い，これも十分なマージンを取ることができない．そのため，マージンを担保する方法として放射線治療に化学療法を併用することでその効果をさらに高めることがしばしば行われている[22]．ただし，こういった症例はきわめて限られるため，臨床試験として行われる際にはN2 diseaseも含めて検討されることが多い．縦隔リンパ節転移がある場合にも，局所のコントロールを向上させる目的と早期に腫瘍を縮小させることにより，遠隔転移をコントロールすることが成績の向上につながるという考え方である．

いくつかの放射線化学療法に引き続いて切除という2相試験が行われている（表3）．対象るために，従来の化学療法単独の後で切除をすと単純に比較をすることは困難であるが概念的認できる治療方法であると考えられる[23, 27～31]ながら比較3相試験が行われたものは一つもな

Ⅲ. 新規抗癌剤を用いた術前化学

90年代の新規抗癌剤は，従来の抗癌剤と比効率・生存率で良好な結果が報告されている．新規抗癌剤を用いた術前治療法がいくつか報告いる（表4）[30, 32]．なかでも，スイスから報告CDDP＋Docによる術前化学療法の成績は全鏡でリンパ節を証明した症例でありながら，83%・MST33ヵ月というすばらしい結果でこの試験では術後彼らはこれを受けて現在，3のCDDP＋Docに続いて手術を行う際に，3目に放射線の追加ありなしという比較試験を行る（SWS-SAKK-16/00）．

Ⅳ. 放射線化学療法に補助的に手術を追加する意義をみる試験[33)]

切除不能の局所進行肺癌に対しては，放射線と化学療法の併用が現在標準治療とされている．切除の可能性については，施設によって見解が分かれる．そこで，切除可能かどうか疑わしい症例に対して放射線化学療法後に手術を追加する意義があるかどうかについて米国で比較試験が行われた．試験の内容は，臨床病期ⅢA非小細胞肺癌を対象とし，まずCDDP＋VP-16を2コースと放射線治療45Gyを同時併用する．その後，手術をするか追加で放射線治療を行うかを無作為割付した．484例が登録された．放射線追加群では治療関連死亡はほとんどみられなかったが，切除群において7%（14例）の治療関連死亡があった（特に片肺全摘症例がそのうち12例を占めていた）．このため，無再発生存では両群間に有意差が認められたが，全体の生存では両群間に差は認められなかった．治療関連死亡の主なものは，肺全摘後のARDSであった．この結果からみると切除の意義はないという結論になるが，肺全摘まで必要としない症例を選択すれば，切除群の予後がよくなる可能性はある．

Ⅴ. 今後の展望

現在非小細胞肺癌に対する種々の分子標的薬が開発されてきており，erlotinib・gefitinib・avastinを周術期に用いる臨床試験が行われている．今後，これら分子標的薬を用いた術前治療の結果が出れば非小細胞肺癌の治療成績が飛躍的に向上する可能性が示唆される．しかし，本邦ではほとんどが未承認薬であり，本邦でこれら分子標的薬を術前に用いることは当分困難であり海外の動向を注視する必要がある．

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REVIEW ARTICLE

**Tatsuo Ohira · Yasuhiro Suga · Yoshitaka Nagatsuka
Jitsuo Usuda · Masahiro Tsuboi · Takashi Hirano
Norihiko Ikeda · Harubumi Kato**

Early-stage lung cancer: diagnosis and treatment

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Introduction

The lung cancer death rate is increasing throughout the world due to increases in numbers of the elderly, increased environmental pollution, and lack of detection in early stages. At our institution, the 5-year survival rate has gradually improved over the past five decades. These results could be due to improvements in therapeutic procedures, including surgery, chemotherapy, radiotherapy, laser therapy, and immunotherapy. Furthermore, the improvement in survival in Japan may be partially due to mass screening for lung cancer mandated by the Health Insurance Act of 1987. The therapeutic results for lung cancer are unsatisfactory. The 5-year survivals of lung cancer patients according to the Japanese Lung Cancer Registry, are shown in Fig. 1.[1] Good results were obtained only in stage I, but in other stages the results were still disappointing. Thus, in order to reduce deaths from lung cancer, it is necessary to detect and treat early-stage lung cancer.

However, there are various problems in the treatment of early-stage lung cancer. Early-stage lung cancers are classified into two categories according to the location of the tumor: central type and peripheral type, and the treatment of each type has specific problems.

In Japan, the criteria of early-stage lung cancer were first proposed about 30 years ago, in 1975. Peripheral-type early-stage lung cancer was defined as a tumor located in an airway more peripheral than the subsegmental bronchi, with the longest dimension of the tumor being 2 cm or less and with no recognized lymph node or distant metastases. In central-type early-stage lung cancer, the tumor is located in a segmental bronchus. In central-type lesions, even if they are early-stage lung cancer, resection of a large volume of lung is generally necessary. This could be a significant factor for pulmonary dysfunction, especially in older patients. In addition, lung cancer, especially the early-stage central type, has a tendency to develop in multiple lesions. In such cases resection is not a valid option for the treatment of all lesions. Therefore, noninvasive therapeutic modalities were required. Laser therapy has been developed for central-type early lung cancer. For the diagnosis of early-stage central-type lung cancer, autofluorescence fiberscopes, bronchofiberscopic echograms, and optical coherence tomography (OCT) have been developed.

As stated above, the improvement of survival in Japan may be partially due to mass lung cancer screening mandated by the Health Insurance Act of 1987. Mass screening for lung cancer by chest computed tomography (CT) was begun in Japan 10 years ago and is now being used in the United States and Europe. Because large numbers of tiny peripheral lung shadows were detected in many of the CT screening pilot trials,[2,3] it is important to establish an internationally accepted definition of peripheral-type early-stage lung cancer.

Therapeutic guidelines for central-type early-stage lung cancer

In Japan, the therapeutic guidelines for lung cancer were established according to evidence-based medicine, with the support of the Ministry of Health, Labor, and Welfare in 2002. In these guidelines, surgical resection and photodynamic therapy (PDT) are recommended for the treatment of central-type early-stage lung cancer.[4]

T. Ohira (✉) · Y. Suga · Y. Nagatsuka · J. Usuda · M. Tsuboi · T. Hirano · H. Kato
Department of Thoracic Surgery, Tokyo Medical University, 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
Tel. +81-3-3342-6111; Fax +81-3-3342-6154
e-mail: tatsuo@rd5.so_net.ne.jp

N. Ikeda
Department of Thoracic Surgery, International University of Health and Welfare, Tokyo, Japan

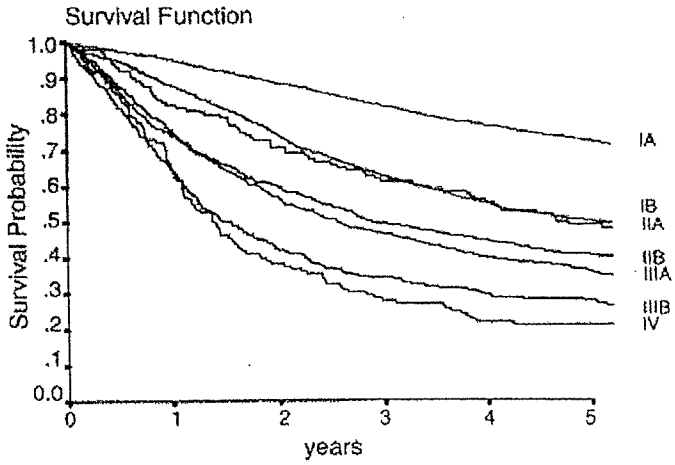

Fig. 1. Survival curves according to clinical (c)-stage. The 5-year survival rates according to c-stage were as follows: 72.1% for IA (n = 2423), 49.9% for IB (n = 1542), 48.7% for IIA (n = 150), 40.6% for IIB (n = 746), 35.8% for IIIA (n = 1270), 28.0% for IIIB (n = 366), and 20.8% for IV (n = 147). There was a significant difference in survival between stages IA and IB (P = 0.0000), between stages IIA and IIB (P = 0.0458), between stages IIB and IIIA (P = 0.0439), and between stages IIIA and IIIB (P = 0.0000). There was no difference in survival between stages IB and IIA (P = 0.4969) or between stages IIIB and IV (P = 0.1577).

Autofluorescence bronchoscopes (AFBs)

Central-type early-stage lung cancer can be cured by noninvasive endoscopic treatment, such as PDT, which has advantages for patients with poor pulmonary reserve; however, the detection of carcinoma in situ (CIS) is a challenge for bronchoscopists. Such lesions show only subtle changes in the bronchial mucosa,[5] and Woolner[6] reported that 60% of CIS lesions showed no macroscopically abnormal findings. This is particularly true with slightly edematous or superficial mucosal changes that can easily be missed, even by experienced bronchoscopists, because they are only a few millimeters thick. Autofluorescence diagnosis is a powerful method to detect macroscopically subtle lesions of the bronchus. Autofluorescence bronchoscopes (AFBs) have been used in leading facilities throughout the world, and the sensitivity for detection of intraepithelial lesions was reported to be 1.5 to 6 times higher than that of conventional white-light bronchoscopy.

Endobronchial ultrasonography (EBUS)

In order to decide indications for PDT, knowledge of the depth of the bronchial tumor is important. Previously, we assessed depth of tumor invasion by the shape of the tumor and loss of bronchus folds. Endobronchial ultrasonography (EBUS) can image the bronchial wall structure in order to assess the depth of bronchial tumor invasion.

Malignant tissues are imaged as hypoechoic areas, and tumor invasion of the cartilage layer is clearly detected. The bronchial wall structure can be imaged as six distinct layers. The cartilage layer is easily identified and can be used to evaluate bronchial wall invasion.

Optical coherence tomography (OCT)

Optical coherence tomography (OCT) is a new modality to detect early-stage lung cancer. OCT can obtain high-resolution, cross-sectional microscopic images of tissue, potentially enabling an optical biopsy to substitute for conventional excisional biopsy. We sought to investigate the capability of OCT to image the microstructure of normal and abnormal bronchial tissue. To assess the depth of bronchial tumor invasion, OCT imaging of the bronchial wall structure was clearer than EBUS, but OCT could detect only the surface of the bronchus.

The OCT system we used was produced by Light Lab Imaging (Boston, MA, USA) and Pentax (Tokyo, Japan). We inserted the OCT catheter via the working channel of the bronchoscope to evaluate the bronchial lumen. The catheter delivers a radial OCT beam and scans circumferentially to generate a transluminal image. In central-type lung cancers, the tumors showed unevenly distributed high backscattering areas and resultant loss of the normal layer structure. We believe that OCT will be able to detect nuclear structure and be used for diagnose is similarly to biopsy in the future.[7]

Possibility of limited resection by video-assisted thoracoscopic surgery (VATS)

The standard therapeutic procedure for peripheral-type early-stage lung cancer is believed to be lobectomy with mediastinal lymph node dissection. However the question has been raised whether lobectomy is really needed for tiny tumors, particularly those less than 1 cm in greatest dimension. There are several reports on limited resection of small lung cancers.[8,9] Some of these results showed satisfactory 5-year survival rates. Clinical trials to clarify the possibility of limited resection are needed for particularly small lung cancers showing ground-glass opacity (GGO), or ground-glass attenuation (GGA). Most of such lesions showed no lymph node metastases, and a 5-year survival of 100% was obtained in patients with such cases who underwent resection. Wedge resection of small lung cancers by VATS without lymph node dissection is one type of minimally invasive surgery. If some types of lung cancer could be shown to be resected by VATS without any increase in local recurrence, this method could become a future standard treatment for peripheral small lung cancers.

Rate of lymph node metastasis of peripheral small nodular cancers

In the past 5 years, 983 patients with lung cancer underwent surgery at our institution. Among them, a total of 159 pa-

tients were studied (Table 1). The tumor size was classified into three categories: 1 cm or less, 1 to 1.5 cm, and 1.5 to 2 cm (47, 49, and 63 patients, respectively). There were 147 pathological N0 patients; lymph node metastasis was recognized in 12 patients (7.5%); this was N1 in 3, and N2 in 9. Table 2 shows the rate of lymph node involvement according to tumor size. In patients with tumors of 1 cm or less, 98% showed no lymph node involvement; however, even in these tiny tumors, 2% showed N2 disease. In tumors between 1 and 1.5 cm, 94% showed no metastasis, but 6% were either N1 or N2. In tumors between 1.5 and 2 cm, lymph node involvement was recognized in 13%.

In this study, the percentages of GGO in tumors were extensively analyzed. We divided tumors into two categories according to how much of the lesion consisted of GGO findings. According to these criteria, 44 tumors showed more than 50% GGO and 115 showed less than 50% GGO. Tumors with a GGO ratio of more than 50% showed no lymph node metastases. On the contrary, all node-positive tumors showed a GGO ratio of less than 50% (Table 3). The relationship between percent GGO area on High Resolution Computed Tumography (HRCT) and the Noguchi classification[10] is shown in Table 4.

Twenty-five of the 44 tumors (57%) showing a GGO component of more than 50% on HRCT were Noguchi type A and B. Seventeen of the 71 tumors (24%) of type C showed more than 50% GGO, and the remaining 54 type C tumors (76%) showed less than 50% GGO. Fifty-three of the 55 (96%) type D, E, and F tumors showed less than 50% GGO. A good correlation between the CT findings and the Noguchi classification was recognized.

The relationship between representative clinicopathological factors and the percent GGO area is shown in Table 5. According to the χ^2 test, the percent GGO area was related to tumor size ($P = 0.0135$) and pathological stage ($P = 0.04$). In particular, a significant relationship with percent GGO was obtained for pathological features including the Noguchi classification ($P = 0.0001$), vascular invasion, and lymphatic invasion.

The overall 5-year survival rate of the patients studied was 88.0%, but it was 96.7% in those with tumors less than 1 cm in diameter, 81.6% in those with tumors between 1 and 1.5 cm, and 84.4% in those with tumors between 1.5 and 2 cm.

The 5-year survival rate was also analyzed according to percent GGO in the lesion. In patients with more than 50% GGO, a 100% 5-year survival rate was obtained, but those with less than 50% GGO had an 83.9% 5-year survival rate.

According to the Noguchi classification, a 5-year survival rate of 100% was obtained in types A and B, with 5-year survivals of 97.4% in type C, 67.1% in types D, E, and F, respectively, which was significantly lower than the results for types A and B and C.

Future surgical procedures for peripheral early-stage lung cancer

Tumors with 100% GGO findings on CT images could indicate suitability for limited surgical resection by VATS. Lesions showing between 50% and 100% GGO may also be indicated for limited resection in tumors less than 2 cm in

Table 1. Patient characteristics

Characteristics	
Age (years)	
Average	63
Minimum	40
Maximum	84
Sex	
Male	67
Female	92
Smoking habit	
Non-smoker	89
Smoker	70
Operative procedure	
Lobectomy	112
Segmentectomy	20
Wedge resection	27

Table 2. Tumor size and nodal status

Tumor size	N0	N1	N2
1.0 cm or less ($n = 47$)	46	0	1
1.0–1.5 cm ($n = 49$)	46	1	2
1.5–2.0 cm ($n = 63$)	55	2	6

Table 4. GGO area and Noguchi classification

GGO%	A, B	C	D, E, F	
More than 50%	25	17	2	44
Less than or equal to 50%	8	54	53	115

Table 5. Relationship between prognostic factors and percent GGO on HRCT

Prognostic factor	χ^2	P value
Sex	0.162	0.687
Tumor size	8.616	0.0135
Pathological stage: I or II–IV	4.168	0.0412
Noguchi classification: A, B, C or D, E, F	14.442	0.0001
Vascular invasion	6.76	0.0093
Lymphatic invasion	5.326	0.0206

Table 3. GGO area and TN status

GGO%	$T \leqq 1$ cm	$1 < T \leqq 5$ cm	$1.5 < T \leqq 2$ cm	
More than 50%	18	16	10	44
Less than or equal to 50%	29 (1)	33 (3)	53 (8)	115 (12)

Numbers in parentheses are numbers of node-positive tumors

diameter, and also, perhaps, in lesions showing between 10% and 50% GGO findings with a tumor size less than 1 cm in diameter. Evaluation of limited resection for small peripheral nodules was reported previously by several researchers.[8,9,11] However, different opinions concerning the modalities used have been reported.[12,13] There are still controversies concerning limited resection of peripheral small lung cancers. A randomized clinical trial by the Lung Cancer Study Group (LCSG) demonstrated the disadvantages of limited resection for T1N0 tumors in relation to lobectomy.[13] Therefore, clinical evidence of the usefulness of limited resection for peripheral early-stage lung cancers should be established. The features of peripheral lung cancers suitable for limited resection without lymph node dissection should be clarified. This will make it possible to determine the optimal CT findings for limited resection.

In our experience, even if the primary lesion was less than 1 cm in size, nodal involvement was confirmed histologically in some patients. Prognostic factors may depend not solely on tumor size but also on the percent GGO area. It is necessary to clarify the findings of CT images of noninvasive cancer by a clinical multicenter study.

Low-dose CT screening for lung cancer

Helical (spiral) CT imaging in the early 1990s provided a promising test for the detection of smaller nodules in the lungs, compared with traditional chest radiography, as images of the chest could be obtained in less than 20 s at a low dose of radiation. It is generally accepted that low-dose CT screening leads to early diagnosis of lung cancer in a high percentage of cases. Based on this evidence, annual CT screening provides for detecting the disease at earlier and presumably more commonly curable stages. The Early Lung Cancer Action Project (ELCAP) showed the great superiority of CT imaging over chest radiographic imaging in identifying cancerous "nodules" in the lungs.[14,15]

Adjuvant chemotherapy for early-stage lung cancer

Recently, some reports have shown significant survival results with adjuvant chemotherapy. The Japan Lung Cancer Research Group (JLCRG) conducted a randomized phase III trial of adjuvant chemotherapy with and without uracil plus tegafur (UFT) after complete surgical resection for stage I adenocarcinoma patients. Subgroup analysis of 263 stage IB patients showed a highly significant result for the UFT arm (5-year survival, 84.9% versus 73.5%; $P = 0.005$).[16]

Conclusions

Good results have been obtained in early-stage lung cancer treatments. Photodynamic therapy (PDT) is suitable for central-type early-stage lung cancer. VATS is a good indication for peripheral-type early lung cancer. Recently, less invasive therapies, such as stereotactic radiation therapy,[17] charged-particle therapy,[18] and microwave coagulation therapy[19] have shown promising results. PDT could be a good modality for peripheral lung cancer, too.[20] The important thing is to find the early-stage lung cancers.

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Analysis of *Epidermal Growth Factor Receptor* Gene Mutation in Patients with Non–Small Cell Lung Cancer and Acquired Resistance to Gefitinib

Takayuki Kosaka,[1,6] Yasushi Yatabe,[2] Hideki Endoh,[6] Kimihide Yoshida,[3] Toyoaki Hida,[3] Masahiro Tsuboi,[4] Hirohito Tada,[5] Hiroyuki Kuwano,[6] and Tetsuya Mitsudomi[1,2]

Abstract

Purpose: Non–small cell lung cancers carrying activating mutations in the gene for the epidermal growth factor receptor (EGFR) are highly sensitive to EGFR-specific tyrosine kinase inhibitors. However, most patients who initially respond subsequently experience disease progression while still on treatment. Part of this "acquired resistance" is attributable to a secondary mutation resulting in threonine to methionine at codon 790 (T790M) of EGFR.

Experimental Design: We sequenced exons 18 to 21 of the *EGFR* gene to look for secondary mutations in tumors with acquired resistance to gefitinib in 14 patients with adenocarcinomas. Subcloning or cycleave PCR was used in addition to normal sequencing to increase the sensitivity of the assay. We also looked for T790M in pretreatment samples from 52 patients who were treated with gefitinib. We also looked for secondary *KRAS* gene mutations because tumors with *KRAS* mutations are generally resistant to tyrosine kinase inhibitors.

Results: Seven of 14 tumors had a secondary T790M mutation. There were no other novel secondary mutations. We detected no T790M mutations in pretreatment specimens from available five tumors among these seven tumors. Patients with T790M tended to be women, never smokers, and carrying deletion mutations, but the T790M was not associated with the duration of gefitinib administration. None of the tumors had an acquired mutation in the *KRAS* gene.

Conclusions: A secondary T790M mutation of *EGFR* accounted for half the tumors with acquired resistance to gefitinib in Japanese patients. Other drug-resistant secondary mutations are uncommon in the *EGFR* gene.

Activating mutations in the gene for the epidermal growth factor receptor (EGFR) are present in a subset of pulmonary adenocarcinomas. Tumors with *EGFR* mutations are highly sensitive to gefitinib and erlotinib, small-molecule EGFR-specific tyrosine kinase inhibitors (1–3). These mutations occur in the tyrosine kinase domain of the *EGFR* gene. Deletion mutations in exon 19 and the substitution of leucine with arginine at codon 858 (L858R) account for ~90% of all these mutations (4). *EGFR* mutations are more prevalent in women, never smokers, patients of Asian ethnicity, and those with adenocarcinoma histology (4). These features are the same as those of patients whose tumors have elevated sensitivity to EGFR-specific tyrosine kinase inhibitors. The response rates of lung cancers with an *EGFR* mutation are as high as 80% (5). Responses are often dramatic, and several reports have shown that patients with *EGFR* mutations survive significantly longer after gefitinib treatment than patients without mutations (6). However, it is also common for patients to show disease progression after presenting with an initial marked response to EGFR-specific tyrosine kinase inhibitors. The mean duration of the initial response is about 3 to 7 months (7, 8).

Recently, it has been reported by two groups that a secondary threonine-to-methionine mutation at codon 790 (T790M) of the *EGFR* gene is related to the acquired resistance to gefitinib and erlotinib (9, 10). Crystal structure modeling has shown that residue T790 is located in the ATP-binding pocket of the catalytic region of EGFR, and it seems to be critical for the binding of erlotinib and gefitinib (9). Substitution of the threonine at codon 790 with a bulkier residue, such as methionine, would result in steric hindrance to the binding of these two drugs. A secondary T790M mutation has been identified in one tumor (9) and in three of six tumors (10) with acquired resistance to gefitinib.

Imatinib is a tyrosine kinase inhibitor specific for BCR-ABL, KIT, and platelet-derived growth factor A, which is used to treat

Authors' Affiliations: Departments of [1]Thoracic Surgery, [2]Pathology and Molecular Diagnostics, and [3]Thoracic Oncology, Aichi Cancer Center Hospital, Nagoya, Japan; [4]Department of Surgery, Tokyo Medical University, Tokyo, Japan; [5]Division of General Thoracic Surgery, Osaka City General Hospital, Osaka, Japan; and [6]Department of General Surgical Science, Graduate School of Medicine, Gunma University, Maebashi, Japan

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Requests for reprints: Tetsuya Mitsudomi, Department of Thoracic Surgery, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. Phone: 81-52-762-6111; Fax: 81-52-764-2963; E-mail: mitsudom@aichi-cc.jp.

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chronic myelogenous leukemia (CML) and gastrointestinal stromal tumor. Analogous secondary mutations in the kinase domains of these genes are considered to constitute one of the mechanisms of acquired drug resistance (11–14). The structural similarity between ABL and EGFR tyrosine kinases is fairly high, and the most common mutation related to acquired resistance is a threonine-to-isoleucine mutation at codon 315 (T315I), corresponding to T790M in the *EGFR* gene (15). In CML, 20 to 30 other mutations of the *ABL* gene have been identified as responsible for acquired resistance to imatinib (12, 16–19), so secondary *EGFR* gene mutations other than T790M are possible (Fig. 1).

Secondary mutations of the *ABL* gene have also been detected in pretreatment samples from some CML patients, although the fraction of mutant cells was very low (16, 20). The existence of a similar mechanism is expected for non–small cell lung cancer. Furthermore, we and others have reported that the T790M mutation of the *EGFR* gene exists as a major mutation independently of gefitinib treatment, although instances are very rare (21, 22).

It has also been reported that *KRAS* mutations are associated with a lack of sensitivity to gefitinib and erlotinib (23, 24). Therefore, it is possible that acquired *KRAS* mutations are also associated with acquired resistance.

In this study, we looked for the T790M mutation and other secondary mutations of the *EGFR* gene in tumors from patients who showed disease progression after presenting with an initial response to EGFR-specific tyrosine kinase inhibitor treatment and in tumors before gefitinib treatment. We also looked for *KRAS* mutations in the same tumors.

Materials and Methods

Patients. Patients with non–small cell lung cancer who initially responded but subsequently experienced disease progression while on gefitinib treatment were defined as having "acquired resistance." A detailed definition of the effectiveness of gefitinib treatment was described in our previous study (25). Briefly, gefitinib treatment is judged to be effective when tumors show a decrease of at least a 30% in tumor diameter in imaging studies or when elevated carcinoembryonic antigen levels decrease to a level less than half the baseline level.

Fourteen tumor samples and 10 corresponding pretreatment tumor samples from eligible patients were obtained according to this definition at the time of diagnosis or treatment. The selection of patients depended only on whether a second tumor sample collected at the time of progression could be obtained. Appropriate approval from the institutional review board and the patients' written informed consent were obtained. Patient characteristics and details of the samples are shown in Table 1. All patients had adenocarcinomas, and the median duration of gefitinib treatment was 367 days (range, 69-921 days). We also analyzed the samples of 52 patients who had been treated with gefitinib for recurrent disease after they had undergone pulmonary resection. This cohort was part of our previous study, and their clinical details are described elsewhere (25).

Subcloning mutational analysis of the EGFR *gene.* Genomic DNA and total RNA (if possible) were extracted from each sample (Table 1). Exons 18 to 21 of the *EGFR* tyrosine kinase domain were amplified using PCR or reverse transcription-PCR (RT-PCR) methods. PCR for genomic DNA was done using AmpliTaq Gold (Applied Biosystems, Foster City, CA) and the following primers: exon 18, 5′-GAGGTGACCC-TTGTCTCTGTGT-3′ (forward) and 5′-CCCAAACACTCAGTGAAACAAA-3′ (reverse); exon 19, 5′-TGCCAGTTAACGTCTTCCTTCT-3′ (forward) and 5′-ATGTGGAGATGAGCAGGGTCTA-3′ (reverse); exon 20, 5′-TGAAACTC-AAGATCGCATTCAT-3′ (forward) and 5′-CATGGCAAACTCTTGCTATCC-3′

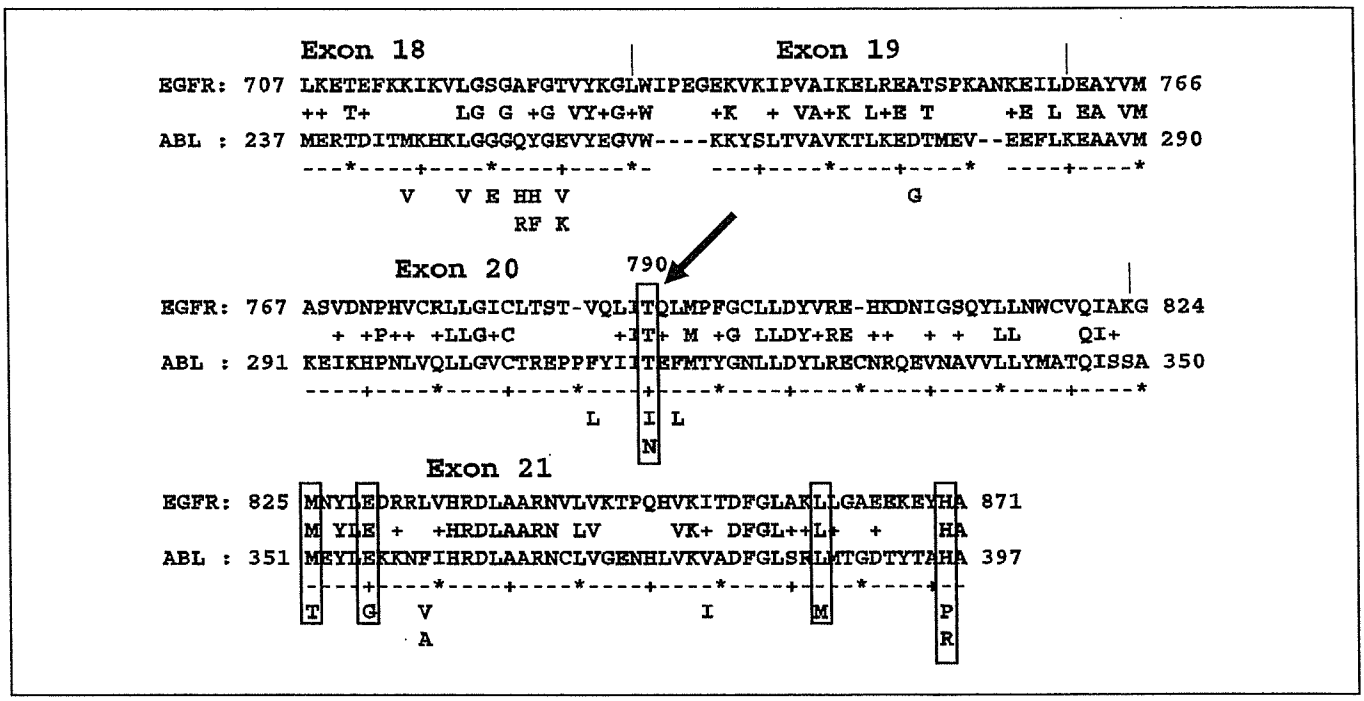

Fig. 1. Structural similarity between EGFR tyrosine kinase and ABL. This amino acid alignment was obtained using basic local alignment search tool, and both sequences were obtained from Genbank (accession nos.: EGFR, NM 005228; ABL, NM 005157). Top line, EGFR; bottom line, ABL. Vertical lines, boundaries between exons. Numbers at each end, codon numbers. Capital letters under the alignment, amino acid changes in ABL that have been reported as acquired imatinib resistance mutations. Square frames, qualifying codons as common codons in EGFR and ABL and as acquired resistance mutant codons in ABL. Arrow, location of codon 790 of EGFR and codon 315 of ABL.

Table 1. Patient characteristics and results of sequencing analysis

Patient no.	Sex	Smoking status	Prior treatment	Gefitinib response	Gefitinib treatment days	Analyzed specimen (state)	Nucleic acid	Activating mutation	T790M mutation	T790M (pre-gefitinib samples)
1	F	NS	S	E	642	LN (Fr)	RNA	Δ2	+	—
2	M	FS	S	E	368	PE (Al)	RNA	Δ3	–	—
3	M	NS	S	E	116	PE (Al)	RNA	Δ1	–	—
4	F	FS	CT	E	599	PE (CL)	RNA	Δ1	–	NA
5	F	NS	CRT	E	921	LU (Al)	RNA	Δ1	+	NA
6	F	NS	None	E	181	PE (Al)	RNA	Δ1	+	—
7	F	FS	CT	E	346	BO (Al)	RNA	Δ1	+	—
8	F	NS	S→CRT	E	623	LN (Al)	RNA	L858R	–	NA
9	M	FS	S	E	915	BR (Fr)	DNA	L858R*	–	—
10	M	FS	S→CRT	NE	69	PE (Al)	DNA	L858R	–	—
11	F	FS	None	E	560	LU (Fr)	RNA	L858R*	+	NA
12	F	NS	CT	E	239	PE (Al)	RNA	Δ1	+	—
13	F	NS	S	E	367	PE (Al)	RNA	L858R	–	—
14	F	NS	CRT	E	235	LN (Al)	RNA	Δ1	+	—

NOTE: Patients 1, 4, and 13 received gefitinib therapy twice. Pretreatment samples from patients 4, 5, 8, and 11 were not available. Patient 10 was defined as not evaluable according to our definition. However, this patient showed a 46% decrease in carcinoembryonic antigen and a marked reduction in pleural effusion on initial treatment before subsequent progression. Therefore, we regarded this case as eligible for this study.

Abbreviations: Al, alcohol fixed; BO, bone metastasis; BR, brain metastasis; CL, cell line; CRT, chemoradiotherapy; CT, chemotherapy; del, deletion; E, effective; F, female; Fr, frozen; FS, former smoker; ins, insertion; LN, lymph node; LU, lung tumor; M, male; NA, not available; NE, not evaluable; NS, never smoker; PE, pleural effusion; RT, radiotherapy; S, surgery; Δ1, del E746-A750; Δ2, del L747-P753 insS; Δ3, del L747-A750 insP.

*Patients 9 and 11 had another point mutation (L833V in patient 9 and R776H in patient 11).

(reverse); and exon 21, 5′-GAGCTTCTTCCCATGATGATCT-3′ (forward) and 5′-GAAAATGCTGGCTGACCTAAAG-3′ (reverse). The PCR conditions were as follows: 1 cycle of 95°C for 11 minutes, 45 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 40 seconds followed by 1 cycle of 72°C for 4 minutes.

RT-PCR for RNA was done with primers 5′-AGCTTGTGGAGCCTCT-TACACC-3′ (forward 1) and 5′-TAAAATTGATTCCAATGCCATCC-3′ (reverse 1) in a one-step RT-PCR setup using Qiagen OneStep RT-PCR kits (Qiagen, Valencia, CA) as described previously (26). RT-PCR conditions were as follows: 1 cycle of 50°C for 30 minutes and 95°C for 15 minutes, 40 cycles of 94°C for 50 seconds, 62°C for 50 seconds, and 72°C for 1 minute followed by 1 cycle of 72°C for 10 minutes.

The PCR products were subcloned using TOPO TA Cloning kits (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Each clone was then directly amplified with the same primers using AmpliTaq Gold and cycle sequenced using BigDye Terminator v3.1/1.1 cycle sequencing kits (Applied Biosystems). Subcloning PCR conditions were as follows: 1 cycle of 95°C for 11 minutes, 45 cycles of 95°C for 50 seconds, 62°C for 50 seconds, and 72°C for 70 seconds followed by 1 cycle of 72°C for 4 minutes.

The sequencing reaction products were electrophoresed using an ABI PRISM 3100 system (Applied Biosystems). Both forward and reverse sequences were analyzed with basic local alignment search tool, and the chromatograms were analyzed by manual review.

Cycleave real-time PCR assay. Details of the cycleave real-time PCR assay have been described previously (27). Briefly, genomic DNA was extracted, and exon 20 of the *EGFR* gene was amplified by real-time quantitative PCR assay on a SmartCycler (TaKaRa, Gifu, Japan) using Cycleave PCR Core kits (TaKaRa) with a T790M-specific cycling probe and a wild-type cycling probe. As few as ~5% of tumor cell molecules could be detected in this assay.

Mutational analysis of the KRAS *gene.* A RT-PCR direct sequence assay was done for RNA, and a cycleave real-time PCR assay was done for DNA. *KRAS* primers for PCR were 5′-GGCCTGCTGAAAATGACTGA-3′ (forward 1) and 5′-TCTTGCTAAGTCCTGAGCCTGTT-3′ (reverse 3). Codon 12 cycling probes and a wild-type cycling probe were used in cycleave real-time PCR assays. Direct sequencing was used to identify codon 12, 13, and 61 mutations.

Results

Detection of secondary mutations in the EGFR *gene or the* KRAS *gene.* For the analysis of secondary mutations, we first amplified exons 18 to 21 of the *EGFR* gene, which include the region homologous to the region of the *ABL* gene that contains all the secondary mutations thus far reported to be responsible for imatinib resistance in CML. All 14 tumors with acquired resistance had activating mutations of the *EGFR* gene, either deletion mutations, including codons 746 to 750 (nine patients), or L858R (five patients). Seven tumors had a secondary T790M mutation (Table 1; Fig. 2).

When we sequenced corresponding tumor samples that had been obtained before gefitinib treatment, the same activating mutations were always present, whereas T790M was not detected in any of the available pretreatment samples (samples for patients 4, 5, 8, and 11 were not available).

Mutant bands for T790M in the sample from patient 7 were as strong as the wild-type bands, and the mutant bands were stronger than the wild-type bands in patient 12 (Fig. 2). However, in most cases, the T790M mutant bands were weaker than the wild-type bands.

Two tumors had another point mutation as well as L858R (L833V in patient 9 and R776H in patient 11). L833 corresponds to F359 of ABL, where a secondary mutation to valine or alanine has been reported in CML (Fig. 1; ref. 12). However, the pretreatment sample of patient 9 revealed that L833V existed before treatment in the same ratio as the L858R band. The ratios of L833V and L858R bands were unchanged

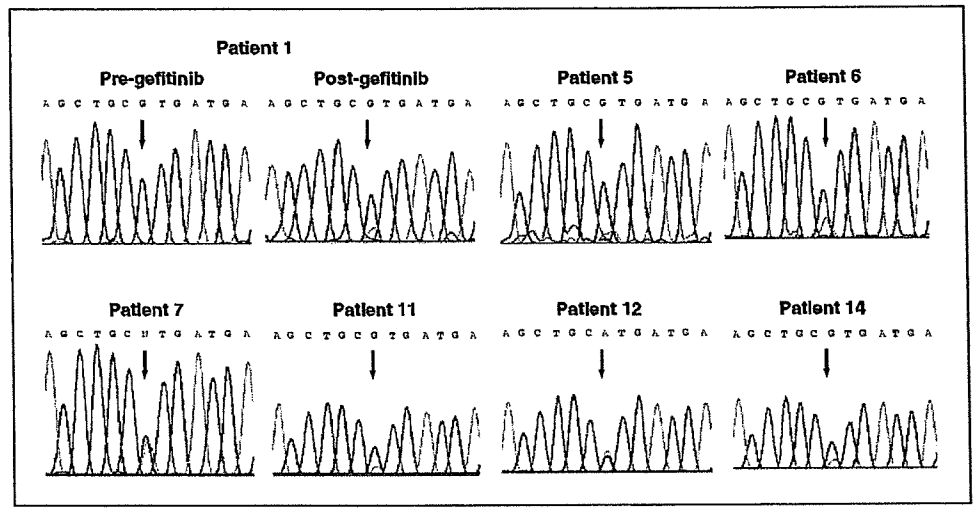

Fig. 2. Sequencing chromatograms for *EGFR* exon 20. Secondary T790M mutations were observed in seven patients. Antisense strands of each chromatogram. Arrows, small peaks of the C→T substitution at nucleotide 2,369 (G→A on the antisense strand), which results in the T790M mutation. This substitution was observed only in posttreatment samples. T790M mutant bands were clearly detected on sequencing chromatograms, except in that of patient 5; in this patient, it was unclear because of artifacts.

before and after gefitinib treatment. Although the T790M mutant band was weaker than the L858R mutant band in patient 11, the intensity of the R776H mutant band was the same as that of the L858R mutant band and both mutations were heterozygous. We considered these point mutations to be primary mutations and not associated with "acquired" resistance.

To increase the sensitivity for the detection of T790M and other possible secondary mutations in the tyrosine kinase domain, each PCR product was subcloned and multiple subclones were amplified and sequenced directly. All the T790M mutations found by sequencing the noncloned PCR products were confirmed by this subcloning method, but no new T790M mutations were detected even when >50 clones were analyzed in samples from patients 2 and 3 (Table 2). Furthermore, we detected no secondary mutations in exons 18 to 21 other than T790M.

The T790M mutations were either present in clones with activating (or sensitizing) mutations or in other clones without activating mutations (Table 2). In three tumors (of patients 1, 5, and 14), T790M was present only in clones with activating mutations, whereas in the remaining four tumors (patients 6, 7, 11, and 12), T790M was present in both clones with and without activating mutations. No tumor carried the T790M mutation only in the wild-type clones. However, four of five T790M mutations were in clones without activating mutations in the tumor of patient 6.

We also looked for mutations in codon 12 (and codons 13 and 61 in RNA samples) in the *KRAS* gene. However, none of the samples from the tumors studied had *KRAS* mutations.

Relationship between T790M mutation and clinical and genetic features. T790M mutations were more frequent in women (women, 7 of 10; men, 0 of 4), who had never smoked (never smoker, 5 of 8; previous smoker, 2 of 6), and with deletion mutations (deletion, 6 of 9; L858R, 1 of 5). There was no difference in the incidence of T790M in the presence or absence of prior chemotherapy (with, 4 of 8; without, 3 of 6; Table 1).

We also compared the duration of gefitinib treatment, which is considered to correlate roughly with the time to progression, with the presence or absence of T790M. However, the median treatment times were almost identical (tumors with T790M, 346 days; tumors without T790M, 368 days; Fig. 3).

Analysis of corresponding tumor tissues before gefitinib treatment in patient 1. To determine whether rare T790M

Table 2. Analysis of acquired mutation using the subcloning method

Patient no.	Activating mutation	Total clones	Activating mutant clones		Wild-type clones	
			With T790M	Without T790M	With T790M	Without T790M
1	Δ2	21	8	10	0	3
2	Δ3	54	0	52	0	2
3	Δ1	51	0	50	0	1
4	Δ1	21	0	13	0	8
5	Δ1	51	3	39	0	9
6	Δ1	47	1	17	4	25
7	Δ1	20	4	5	1	10
8	L858R	18	0	14	0	4
9	L858R	20	0	14	0	6
10	L858R	20	0	5	0	15
11	L858R	21	5	10	1	5
12	Δ1	23	11	9	1	2
13	L858R	21	0	8	0	13
14	Δ1	19	7	8	0	4

mutant clones existed before gefitinib treatment, we analyzed the corresponding tumor tissues of patient 1, whose tissue after gefitinib treatment had a secondary T790M mutation. Tumor tissue was obtained at the time of operation. PCR products from the tumor before gefitinib treatment were subcloned, and 103 subclones were amplified and sequenced directly. However, at this sensitivity, we detected no clone carrying the T790M mutation. Among 103 clones, 92 (89%) had activating deletion mutations, suggesting that the mutant allele was amplified before gefitinib treatment. The incidence of clones with deletional mutations was similar (18 of 21, 85%) in a cervical lymph node taken after gefitinib resistance had developed.

To further explore of possible association of T790M with metastatic spread, we looked for the T790M mutation in hilar and mediastinal lymph nodes with metastases dissected at the time of surgery. Genomic DNA was extracted from lymph nodes from four stations (aortopulmonary, ascending aorta, main bronchus, and intrapulmonary) and analyzed using cycleave real-time PCR. However, we detected no T790M mutations.

Analysis of tumors for T790M before gefitinib treatment in 52 patients who were treated with gefitinib. The possible presence of T790M at a low frequency in tumors before gefitinib treatment might affect the tumor response or the time to progression after gefitinib treatment. In a previous study, we sequenced exons 18 to 23 of the *EGFR* genes of 52 patients who had been treated with gefitinib for recurrent disease after they had undergone pulmonary resection. None of them had the T790M mutation. Here, we used a cycleave real-time PCR assay, which is more accurate analysis than normal sequence, to investigate whether rare T790M mutant cells were present. However, we detected no T790M mutations in these 52 tumors.

Discussion

We studied 14 tumors with acquired resistance to gefitinib for secondary mutations occurring in the *EGFR* tyrosine kinase domain. Seven of the 14 tumors had a secondary T790M mutation, an incidence consistent with those of previous studies (9, 10). Whereas clones with activating mutations (deletion or L858R) might well have been eliminated by selection pressure during gefitinib treatment, those clones were always present in tumors that developed acquired resistance. In most cases, clones with the T790M mutation were not predominant.

The T790M mutations occur more frequently in women who had never smoked and who had a deletion-type mutation. Time to progression did not differ between tumors that acquired secondary T790M mutations and those that did not. However, these tendencies require careful interpretation because of the number of samples was small.

In a previous report, Kobayashi et al. (9) showed that the T790M mutation was observed with either wild-type or deletion mutation sequences, whereas Pao et al. (10) showed that both the T790M and L858R mutations were in the same allele. Our data showed that three samples had the T790M mutation only in the clones with activating mutation and four samples had the T790M mutation in the clones with and without activating mutation, whereas the most of T790M mutation was in the clones with activating mutation, except for the samples of patient 6. It is possible that this could result from a PCR error or DNA repair error at the subcloning step. Bell et al. (28) have reported that artifactual PCR-generated allelic separation occurred with probability of ~30% in their analysis. However, it is also possible that the T790M mutation occurs in both alleles or that tumor heterogeneity exists.

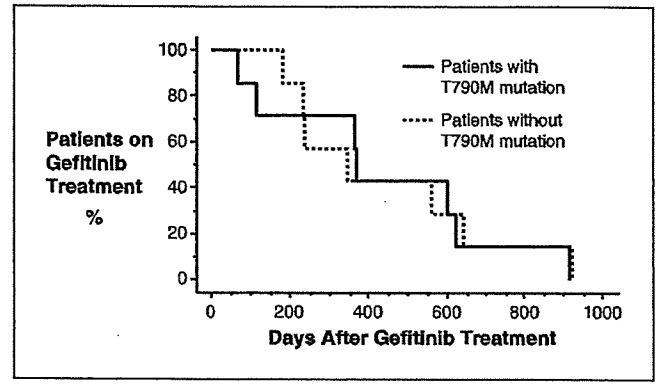

Fig. 3. Effect of the T790M mutation on the length of gefitinib treatment. The length of gefitinib treatment was considered to be roughly related to time to progression. Median treatment times were almost identical in both the presence and absence of the T790M mutation.

In CML, 20 to 30 mutations in the *ABL* gene are responsible for acquired resistance to imatinib. Many types of mutations have been detected, and there are four distinguishable clusters (P-loop, T315, M351, and A-loop; ref. 29). Furthermore, secondary mutations in the ABL kinase domain are found in 50% to 90% of patients (29), many more than in patients with non-small cell lung cancer. We detected no novel mutations in the *EGFR* gene other than T790M. Two tumors had another point mutations together with L858R, L833V, or R776H. We considered these point mutations to be primary mutations and not associated with acquired resistance. However, these conclusions were based only on sequencing and subcloning methods, and we have no evidence of the functional effects of these mutations. There may be differences in the mechanisms of acquired resistance between non-small cell lung cancer and CML.

We previously reported that, in a series of 397 unselected patients with non-small cell lung cancer who had undergone surgery, 2 female patients with no history of smoking had L858R plus T790M mutations (21). Because these patients were not treated with gefitinib, T790M might well have conferred a growth advantage. These tumors were aggressive and later developed recurrent disease. One was treated with gefitinib but was refractory to treatment. A similar case was reported by another group (22). Inspired by this observation and because the secondary mutations related to imatinib resistance in CML were detected at low frequencies (0.01-0.9%) in pretreatment samples (16, 20), we attempted to detect minor clones with the T790M mutation in samples before gefitinib treatment. However, we could not detect the T790M mutation by assays that can detect mutant cells if there is about 1% to 5% at least. It remains unclear whether a more sensitive method would have detected rare clones with the T790M mutation in our samples.

Why tumors with T790M mutant cells acquire resistance to gefitinib despite the fact that mutant band for the T790M

mutation was almost always weaker than wild-type band remains unclear. It is possible that cells with the T790M mutation preexist at a very low frequency and gradually increase during gefitinib treatment by clonal selection as in cases of CML (16). It is also possible that amplification of the activating mutant allele occurs in resistant tumors and parts of them have the T790M mutation. Another possibility is that multiple coexisting mechanisms, including the T790M mutation, cause acquired resistance cooperatively or independently. A recent study suggested that increased internalization of ligand-bound EGFR is one of the mechanisms underlying acquired gefitinib resistance (30). It is also likely that *EGFR* gene amplification (31) by alteration of downstream molecules, such as AKT (32), might play a role in the acquisition of resistance to gefitinib.

Mutations in *KRAS* are associated with a lack of sensitivity to gefitinib and erlotinib (23). We looked for *KRAS* mutations because of the possibility that acquired *KRAS* mutations are associated with acquired resistance. There were no *KRAS* mutations in any tumor. The same finding has been reported in a previous study (10), suggesting that *KRAS* mutations are not associated with acquired resistance.

In conclusion, half of tumors with acquired resistance to gefitinib had secondary T790M mutations. No novel mutations in the *EGFR* gene were present in contrast to CML.

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Surgery for Bronchioloalveolar Carcinoma and "Very Early" Adenocarcinoma: An Evolving Standard of Care?

Valerie W. Rusch, MD, Ryosuke Tsuchiya, MD, PhD,† Masahiro Tsuboi, MD,‡ Harvey I. Pass, MD,§ Dominique Grunenwald, MD,|| and Peter Goldstraw, FRCS¶*

Abstract: Lobectomy and mediastinal lymph node dissection is the standard surgical management of early stage non-small cell lung cancer (NSCLC) because more limited resections have been associated with a higher risk of local recurrence. Nevertheless, recent lung cancer screening studies have led to the detection of an increasing number of "very early" NSCLC (defined as less than 2 cm in size) and of good-prognosis histologic subtypes, bronchioloalveolar carcinoma (BAC), and adenocarcinoma (AC), mixed subtypes that are potentially appropriate for sublobar resection. The precise indications for sublobar resection remain unclear and are the subject of ongoing clinical trials, but it seems that very early, peripherally located, node-negative AC of a predominantly BAC pattern may be adequately treated in this manner. Multifocal AC and BAC, either synchronous or metachronous, are also effectively treated by complete resection, using limited resections whenever possible. The pneumonic form of BAC, the rarest variant of this disease spectrum, continues to have a poor prognosis despite complete resection. Very limited experience suggests that lung transplantation leads to prolonged survival in highly selected patients with this histologic subtype. To improve our management of very early AC, much more information is needed about the molecular abnormalities of AC and their relationship to clinical outcomes.

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During the past decade, thoracic surgeons have been confronted with demographic and pathological shifts in the group of non-small cell lung cancers (NSCLC) that are potentially resectable.[1] In many countries, adenocarcinoma (AC) has become the most common NSCLC histology. The proportion of women with lung cancer has increased dramatically; in some institutions, half of all patients are female. The number of patients who have never smoked or who have minimal past tobacco exposure is also increasing, especially in North America, because of tobacco control efforts. The widespread use of computed tomography (CT) for lung cancer screening has also led to increased detection of "very early" NSCLC, generally defined as tumors that are 2 cm or less in size, which are usually ACs of mixed subtype or bronchioloalveolar carcinomas (BAC) and which tend to have an indolent clinical behavior.

These epidemiologic shifts have led thoracic surgeons to reexamine the accepted tenets of surgical management of early-stage NSCLC. As part of the November 2004 symposium on BAC, which is the subject of this supplement, a group of thoracic surgeons were asked to review the current management of BAC and very early ACs, focusing especially on the role of sublobar resection. This paper summarizes the discussions held at the symposium and provides updated information on relevant clinical trials.

PATHOLOGICAL CLASSIFICATION OF AC: RELEVANCE TO SURGICAL MANAGEMENT

BAC has long been recognized as a distinct form of AC associated with a favorable prognosis. In 1989, the North American Lung Cancer Study Group (LCSG) reviewed 1635 patients who had undergone resection of AC, 235 of whom had BAC. Resectable BAC occurred more frequently in never-smokers, was diagnosed at an earlier disease stage, and was associated with a better survival rate than invasive AC.[2] During the last 40 years, improved understanding of the pathology of lung AC has prompted substantial changes in the histologic subclassification by the World Health Organization (WHO), which are summarized by Travis et al.[3] in their report from the pathology panel of this symposium (Table 1). From 1967 to 1999, multiple subcategories were added to reflect increasing knowledge about the histologic heterogeneity of AC. Significant changes in the 1999 WHO classification included the addition of atypical adenomatous hyperplasia (AAH) as a preinvasive lesion for lung AC, and the requirement that all BACs demonstrate pure lepidic growth without invasion of stroma, blood vessels, or pleura. In 2004, AC mixed subtype was moved to the top of the list of subcategories in recognition that this is now the most common subtype.[4]

In 1995, Noguchi proposed a six-tier histologic subclassification (types A through F) for small ACs of the lung, recognizing the excellent prognosis associated with BACs (with a purely lepidic growth pattern), the adverse prognostic

*Memorial Sloan-Kettering Cancer Center, New York, New York; †National Cancer Center Hospital, Tokyo, Japan; ‡Tokyo Medical University, Tokyo, Japan; §New York University Medical Center, New York, New York; ||Hôpital Tenon, Paris, France; and ¶Royal Brompton Hospital, London, United Kingdom.

Address for correspondence: Valerie W. Rusch, M.D., Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; E-mail: ruschv@mskcc.org

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TABLE 1. History of Lung Adenocarcinoma Subclassification According to the World Health Organization

1967	Bronchogenic
	Acinar
	Papillary
	Bronchioloalveolar
1981	Acinar adenocarcinoma
	Papillary adenocarcinoma
	Bronchioloalveolar carcinoma
	Solid carcinoma with mucus formation
1999	Acinar
	Papillary
	Bronchioloalveolar carcinoma
	Nonmucinous
	Mucinous
	Mixed mucinous and nonmucinous
	Solid adenocarcinoma with mucin
	Adenocarcinoma with mixed subtypes
	Variants
	Well-differentiated fetal adenocarcinoma
	Mucinous (colloid) adenocarcinoma
	Mucinous cystadenocarcinoma
	Signet-ring adenocarcinoma
	Clear-cell adenocarcinoma
2004	Adenocarcinoma, mixed subtype
	Acinar adenocarcinoma
	Papillary adenocarcinoma
	Bronchioloalveolar carcinoma
	Nonmucinous
	Mucinous
	Mixed nonmucinous and mucinous or indeterminate
	Solid adenocarcinoma with mucin production
	Fetal adenocarcinoma
	Mucinous (colloid) adenocarcinoma
	Mucinous cystadenocarcinoma
	Signet-ring adenocarcinoma
	Clear-cell adenocarcinoma

From Travis WD, Garg K, Franklin WA, et al. Evolving concepts in the pathology and CT imaging of lung adenocarcinoma and bronchioloalveolar carcinoma. J Clin Oncol 2005;23:3279–3287. Used with permission.

TABLE 2. Noguchi's Histology Typing of Small Adenocarcinoma of the Lung

Type	Description
A	Localized bronchioloalveolar carcinoma
B	Localized bronchioloalveolar carcinoma with foci of collapse of alveolar structure
C	Localized bronchioloalveolar carcinoma with foci of active fibroblastic proliferation
D	Poorly differentiated adenocarcinoma
E	Tubular adenocarcinoma
F	Papillary adenocarcinoma with compressive and destructive growth

From Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. Cancer 1995;75:2844–2852. Used with permission.

importance of central fibrosis in BACs, and the pathologic heterogeneity of invasive ACs (Table 2).[5] Although the 2004 WHO classification is the internationally accepted system, Noguchi deserves credit for an early attempt to refine the classification and to correlate it with clinical outcomes. As discussed below, the Noguchi system is still used by Japanese investigators to select patients for sublobar resection in ongoing clinical trials. More recently, Noguchi showed that these histologic subtypes have corresponding molecular abnormalities.[6] Areas of histologic types A, B, and C extracted by microdissection from resected ACs were examined by multiplex PCR-LOH and were found to have a progressive rise in the incidence of allelic losses. Deletions of 3p, 17p, 18q, and 22q increased significantly from types A to C, consistent with a model of malignant progression.

Several Japanese studies now confirm that the histologic subtype correlates with CT findings and clinical outcome.[3,7,19] The results of Kodama exemplify these investigations (Table 3). Taken as a whole, these studies suggest that: 1) pure ground-glass opacities (GGO) on CT usually represent BAC without any areas of invasive AC, whereas lesions that show both GGO and solid components on CT (part solid, part nonsolid) are mixtures of BAC and invasive ACs; and 2) small (less than 2 cm in size) tumors with >50% GGO are associated with a 100% chance of being node negative, have an excellent chance of long-term survival after treatment, and probably can be managed by limited resection rather than lobectomy. However, the appropriateness of limited resection for part solid/part nonsolid lesions is unclear and is the subject of clinical trials in Japan. Tumors that are more than 50% GGO on CT seem to have a better prognosis and may potentially be managed by sublobar resection, but preoperative high-resolution CT and intraoperative frozen-section analysis still do not always accurately identify tumors that have a poorer prognosis. Our uncertainties with respect to the optimal surgical management of these lesions reflect the highly variable presentation and behavior of lung ACs, the limitations of CT findings in predicting pathologic findings, and our lack of knowledge of the histologic and molecular features that predict a poor prognosis.

TABLE 3. Prognosis in Relationship to Appearance (% GGO)

	GGO < 50%	GGO > 50%	*p*
Patients	52	52	—
Size	13.7	12.3	0.09
Node involvement	8	0	0.01
% local resection	50%	70%	0.001
Relapse	9	0	—
DFS	72%	100%	—

GGO, ground-glass opacity; DFS, disease-free survival.

Adapted from Kodama K, Higashiyama M, Yokouchi H, . Prognostic value of ground-glass opacity found in small lung adenocarcinoma on high-resolution CT scanning. Lung Cancer 2001;33:17–25. Used with permission.

RELATIONSHIP OF TUMOR SIZE TO TUMOR STAGE: SURGICAL IMPLICATIONS

In NSCLC, the size of the primary tumor is known to correlate with the likelihood of lymph node metastases and, therefore, to influence consideration of sublobar resection. The frequency of nodal disease in very early NSCLC has been studied extensively.[20 32] Although lymph node involvement is relatively uncommon in small AC, approximately 10% of tumors that are 1 cm or smaller and 20% of tumors that are 1 to 2 cm in size have nodal metastases (Tables 4 and 5). Relative to AC, squamous cell carcinomas less than 2 cm in size seem to be associated with a lower risk of nodal disease.[20] These findings complicate the selection of patients for limited pulmonary resection because we do not fully understand which patients with very early lung AC may have disease in the intralobar lymphatics or regional nodes. A better understanding of the molecular features in early AC and their relationship to clinical outcome is needed to allow accurate decisions about the use of sublobar resection.

LOBECTOMY VERSUS SUBLOBAR RESECTION: CURRENT KNOWLEDGE AND INVESTIGATIONS

A prospective randomized multicenter trial reported by the LCSG in 1995 established lobectomy as the standard approach to resection for T1N0 NSCLC (LCSG trial 821). Sublobar resection, either wedge resection or segmentectomy, for carefully selected patients who had thorough intraoperative evaluation of the extent of the primary tumor and of the N1 and N2 lymph nodes, was associated with a tripling of the local recurrence rate and a 30% increase in the overall death rate. Within the T1 stage category, tumor size did not seem to influence the risk of recurrence, but the numbers of patients who had tumors less than 2 cm in size were small.[33] The increasing incidence of very early NSCLC seen in thoracic surgical practice, primarily via CT screening for lung cancer,[1] has reopened the debate about the use of sublobar resection. This debate is especially relevant to BAC and to some AC of mixed subtype because of their indolent clinical behavior and known propensity for multifocality. Patients with these AC histologic subtypes often have synchronous or metachronous primary tumors that are best managed by resection. Preservation of lung function through the proper use of limited resection can be a critical aspect of achieving prolonged survival and maintaining patients' functional capacity.[34 36] Several retrospective studies and prospective clinical trials suggest that the sublobar resection may be an appropriate operation for very early AC.[11,13,37 40] The parameters that currently seem to allow proper selection of patients for limited resection include tumor size (less than 2 cm and especially 1 cm or less) in combination with tumor histology (BAC or AC, mixed subtype with 50% or greater BAC component or AC, Noguchi types A or B), peripheral tumor location, and absence of N1 or N2 disease based on thorough intraoperative staging. The presence of GGO or of part solid, part nonsolid appearance on CT reflects these tumor characteristics. In ways that are not yet fully understood (aside from the presence of EGFR mutations in some tumors), these clinical and pathologic features represent tumors that most likely have an indolent biological behavior. The adequacy of wedge resection versus anatomical resection via segmentectomy remains undefined, although segmentectomy has been favored in Japanese studies because it provides an optimal deep margin of resection and removes the local lymphatic bed associated with the primary tumor.[39]

Japanese investigators have sought to confirm these selection criteria for sublobar resection through prospective multicenter clinical trials. JCOG trial 0201 (Figure 1), reported at the 2006 meeting of the American Society of Clinical Oncology (ASCO), enrolled patients with clinical

TABLE 4. Prevalence of Nodal Disease in Solid Nodules <2 cm in Size

	n	% Positive Nodes	% N2
Naruke (1993)[23]	287	40	50
Asamura (1996)[24]	174	20	60
Konaka (1998)[25]	171	17.5	66
Takizawa (1998)[26]	157	17	NS
Sugi (1998)[27]	115	19	66
Wu (2001)[28]	136	22	NS
Okada (2003)[29]	265	18	55
Nonaka (2003)[30]	46	28	70
Average		23	

NS. not stated.

TABLE 5. Prevalence of Nodal Disease in Solid Nodules 1 cm or Less in Size

	n	Patients with Positive Nodes (%)
Naruke (1993)[23]	20	8 (16)
Oda (1998)[31]	22	0 (0)
Konaka (1998)[25]	19	0 (0)
Ohta (2001)[20]	11	4 (4)
Miller (2002)[32]	100	7 (7)
Average		9

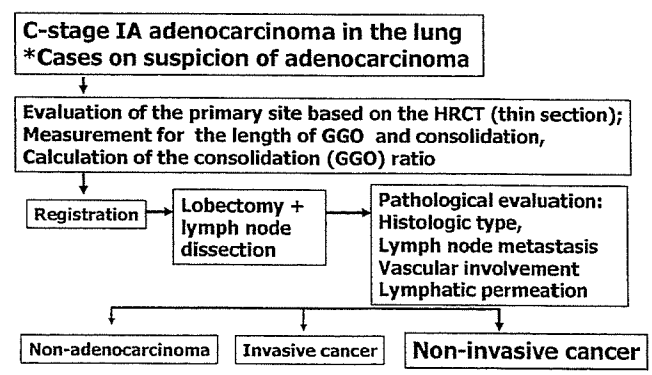

FIGURE 1. Schema for JCOG 0201 trial.

stage IA AC.[41] The primary endpoint was to determine the specificity of high-resolution CT (HRCT) in diagnosing noninvasive AC, using the final pathologic findings as the reference standard. A pathological noninvasive AC was defined as a tumor with no lymph node metastases or lymphatic or vascular invasion. Preoperative evaluation included HRCT to assess the presence of GGO and to calculate the ratio of GGO to solid component of the tumor. Patients then underwent lobectomy and mediastinal lymph node dissection. Final pathological findings were compared with the HRCT features to determine whether the CT could be used to select patients appropriately for sublobar resection. Of the 811 patients enrolled, 545 eligible patients had undergone lobectomy and central data review at the time of the ASCO presentation. Comparison of the CT with the pathological findings showed that HRCT had a specificity of 98.3% but a sensitivity of only 24.7% for the diagnosis of noninvasive AC.

The results of JCOG 0201 have been utilized to develop two new prospective trials. Patients found to have AC 2cm or less in size that are predominantly GGO by HRCT (solid component less than 25% of entire tumor) will be entered on a single arm Phase II trial testing the use of wedge resection for these highly curable indolent tumors. Patients found to have AC 2cm or less in size that have a larger solid component on HRCT (more than 25% but less than 100% of the entire tumor) will be eligible for a prospective randomized comparing lobectomy to limited resection (Figure 2). These trials might also help define which tumors do not require lymph node dissection or sampling, although this is not a planned study endpoint. At the current time lymph node sampling or systematic nodal dissection (SND) remains a key part of accurate tumor staging.[42]

In North America, the Cancer and Leukemia Group B (CALGB), in collaboration with the American College of Surgeons Oncology Group (ACOSOG), is planning a prospective randomized trial comparing lobectomy versus limited resection (wedge or segmentectomy) for patients with AC 2 cm or less in size. This trial does not incorporate the nuanced radiological and histologic selection criteria used in Japanese studies, depending instead on simple size criteria and the basic diagnosis of AC. Designed to reproduce the LCSG 821 trial, but with a focus on smaller tumors, the CALGB trial uses intraoperative assessment of tumor size, tumor location, and nodal involvement, followed by randomization to lobectomy or limited resection. Because of the large numbers of patients and long follow-up time required to identify a survival difference between these two resectional approaches, results from this trial will probably not be available for about 8 years.

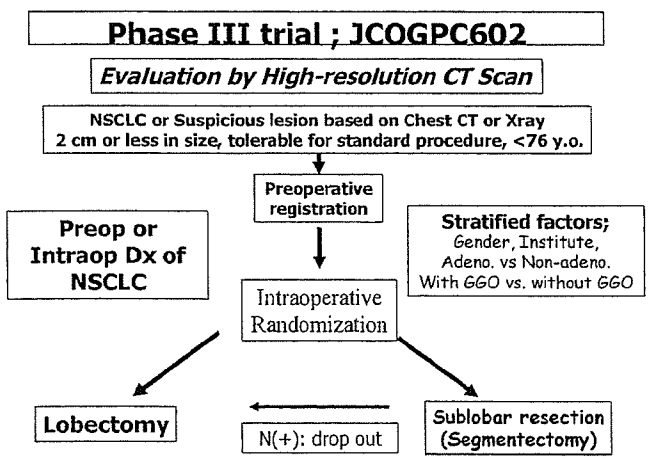

FIGURE 2. Schema for JCOGPC 602 trial.

MANAGEMENT OF THE PNEUMONIC FORM OF BAC: RESECTION, SYSTEMIC THERAPY, OR TRANSPLANTATION?

Most BAC or AC, mixed subtype present as either a single nodule or as multiple lung nodules (synchronous or metachronous) that behave in an indolent manner and are best managed surgically.[34,36,43] The least common variant of this BAC-AC disease spectrum is generally termed the pneumonic form because it presents as a progressive lobar consolidation with mucinous AC filling the alveolar spaces. Resection does not seem to alter the very poor prognosis of this disease, which inevitably progresses to consolidation of both lungs and death from respiratory failure.[34,43] Systemic therapy has also been relatively ineffective in this disease. Thus, most surgeons are reluctant to consider pulmonary resection for this biologically aggressive form of AC. Lung transplantation has been suggested as a potential treatment option. First reported by Zorn et al., lung transplantation in nine patients (single lung in two and bilateral transplants in seven patients) was associated with a poor outcome.[44,45] Only two patients survived long term, whereas the other patients experienced cancer recurrence in the transplanted lungs. More recently, the Toronto group reported their experience with transplantation in 29 patients.[46] Five-year survival was 51%, and recurrence developed in 13 of the transplanted lungs. Although transplantation was performed for advanced multifocal BAC, it is not entirely clear how many of these patients truly had the pneumonic form of mucinous AC. Thus, lung transplantation potentially remains an option for selected patients, but it is associated with a significant risk of recurrent disease and requires further study.

SUMMARY

Lobectomy and lymph node sampling or systematic nodal dissection remain the standard surgical treatment for patients with early stage NSCLC. However, limited resection may be an appropriate option for patients with very early AC and BAC based on tumor size, location, and relative proportion of BAC to AC. Very small BAC are probably appropriately treated by limited resection. Accurate criteria for selecting patients for limited pulmonary resection await the results of ongoing clinical trials and an improved understanding of NSCLC biology in relationship to clinical outcome.

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A．非小細胞癌

1．外科的治療

病変が局所にとどまり遠隔転移をもたない非小細胞肺癌の治療においては，手術が局所療法として最も的確な治療効果を期待されている．肺癌患者に対する手術の適応として，①患者が肺切除に耐え得るかどうか，②外科切除後の患者の期待される予後はどうかの両面から検討する必要がある．生理学的手術適応として，呼吸機能，年齢が，腫瘍学的手術適応として，組織型（非小細胞肺癌，小細胞肺癌），臨床病期（TNM）が判断因子となる．外科治療の基本術式は，原発巣を含む肺葉切除とそれに伴う領域リンパ節の郭清である．したがって，実地医療においては，個々の症例においてその病期における切除成績に加えて，外科治療の術後死亡率および合併症の発生頻度と予想される予後とを考え合わせて外科治療の適応および術式の検討を行い，耐術能のある症例についてはインフォームドコンセントのもとに手術を計画するという態度を要する．ここでは，外科切除が標準的治療の主役を担うであろうⅠ期からⅢA期非小細胞肺癌 non-small cell lung cancer (NSCLC) の病期別治療戦略[1,2]とその治療成績について述べる．

1．Ⅰ期非小細胞肺癌に対する治療

A．自然史

治療選択を論ずる前に，治療しなかったらどうなるか，すなわち「自然史」については本来認識しておくべきかもしれない．しかし，実際にはⅠ期肺癌と診断された大半の症例が何らかの治療的介入を受けるので，その把握は困難である．本邦からは，Sobue らが臨床病期Ⅰ期の非手術例の検診発見例 42 例の生存期間中央値 25 カ月，症状発見例 27 例の生存期間中央値 13 カ月と報告しているが，対象は無治療例以外にも化学療法放射線治療例が含まれている[3]．欧米からは 20～50 例程度の検討からⅠ，Ⅱ期非小細胞肺癌の生存期間中央値は 14～17 カ月程度で，Ⅰ期とⅡ期に有意差を認めなかったと報告されている[4]．

B．治療成績と標準的戦略

Ⅰ期 NSCLC の術後 5 年生存率は臨床病期 IA 期（cT1N0M0）では 72%，IB 期（cT2N0M0）では 50%，病理病期 IA 期（pT1N0M0）では 79%，IB 期（pT2N0M0）では 60%であり[5]，手術以外の治療法との直接の比較試験は存在しないが他の治療法との差異は明らかであり，機能的に耐術可能な場合，Ⅰ期 NSCLC には外科治療が標準的治療戦略である．一方，医学的あるいは社会的・個人的な理由で手術のできないⅠ期 NSCLC には，根治的放射線単独治療の適応があり標準的に推奨される．

C. 手術術式

機能的に耐術可能な場合，I期NSCLCに対する標準的術式は原発巣を含む肺葉切除と系統的リンパ節郭清である．リンパ節郭清については，現時点で予後改善への寄与は不明であるが，正確な病期診断のために必要不可欠とされている．

cT1N0M0のうち症例選択によっては，区域切除や楔状切除，部分切除などの縮小手術が肺葉切除と同等な予後を期待できる可能性があるが，現時点では標準的に行うよう勧めるだけの根拠が明確でない．米国のLung Cancer Study Group（LCSG）で行われたT1N0のNSCLCにおける肺葉切除と縮小手術のランダム化比較試験では，両群の生存期間に統計学的有意差は認められなかったものの，局所再発率が肺葉切除群で5%，縮小手術群で15%と，縮小手術群で有意に高い再発率であった[6]．その一方で，手術死亡，術後合併症，術後呼吸機能は，両群間で差を認めなかった．これらの結果から，縮小手術は合併症などのために肺葉切除が困難な症例で，完全切除が可能なものに限って選択されるべきであると考えられている．また，腫瘍径とリンパ節転移頻度の関係から，腫瘍径2 cm以下の末梢小型肺癌のリンパ節転移陽性率は15～20%程度と報告されており，単純に腫瘍径を指標とした縮小手術の妥当性には疑問が残る[7]．ただし，高分解能CTの導入などによる診断精度の向上に加え，胸腔鏡手術あるいはその周辺機器の発達により，諸外国に比べても肺癌が従来より小型かつ早期に発見される傾向が強まり，また縮小手術によって良好な成績が報告されるようになった．Kodamaらは，T1N0M0肺癌の縮小手術群と肺葉切除群を比較し，積極的な区域切除施行例46例の5年生存率93%であり，肺葉切除例77例の5年生存率88%と有意差を認めず（p=0.86），多変量解析においても術式は予後因子とはならなかったと報告した[8]．Yoshikawaらは，腫瘍径2 cm以下の肺癌に対して術中リンパ節迅速診断を用いた区域切除（extended segmentectomy）を55例に施行した前向き研究を行い，全死による5年生存率81.8%，癌死による5年生存率91.8%という良好な結果を報告している[9]．このように，I期肺癌において症例選択によっては縮小手術により良好な治療成績が得られる可能性が示されており，今後小型肺癌に対する縮小手術の妥当性を検証するランダム化比較試験が必要である．現在，本邦の日本臨床腫瘍グループ Japan Clinical Oncology Group（JCOG）と米国のCancer And Leukemia Group B（CALGB）の双方でほぼ同様の臨床第III相試験が計画中（図6-1）であり，この試験の完遂と結果が待たれる．

D. 術前・術後補助療法

1）術前補助療法

IB期NSCLCに対する術前補助（化学）療法は，依然探索的治療である．

現時点では，I期NSCLCに特化した術前補助療法の臨床試験の結果は報告されていない．

IB～II期といった比較的早期の非小細胞肺癌症例に対する術前化学療法については，フランスのグループの試験のサブセット解析からその有用性が示唆され[10]，米国をはじめ，世界中でこれらの病期に対する術前化学療法の意義を問う試験が，手術単独をコントロール群として行われていた（表6-1）．しかし，スペインのNATCHトライアルを除く多くの比較試験が，症例集積不良と標準治療（コントロール）の変更，すなわち後述する術後補助化学療法が標準的治療戦略に組み込まれるべきことが明らかになったことを受けて中止された．SWOG9900（BLOT or knot）では，IB～IIIA（T3N1）期非小細胞肺癌例を対象にカルボプラチン＋パクリタキセルの化学療法を術前に3コース

図 6-1　JCOG 肺がん外科グループで計画中の小型 NSCLC に対する縮小手術の妥当性に関する phase III study デザイン（案）

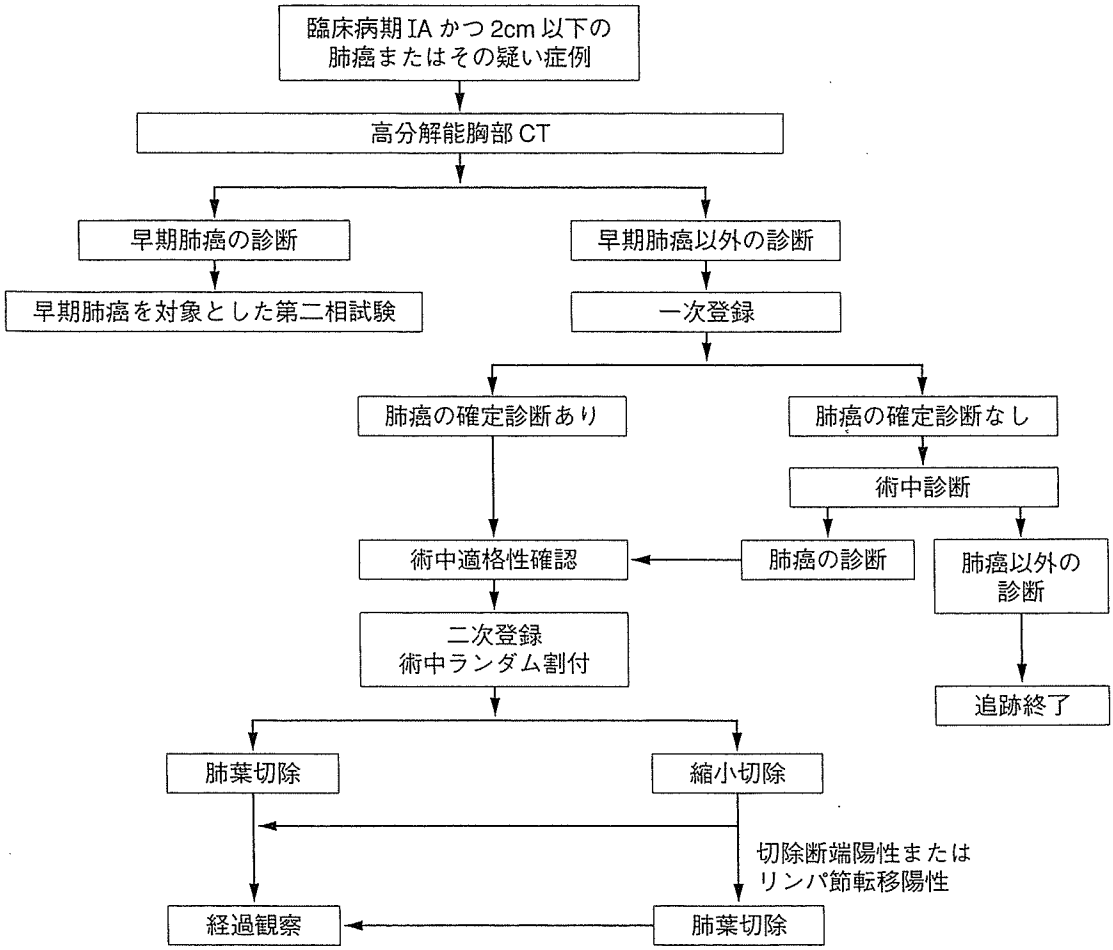

表 6-1　局所限局 NSCLC における術前化学療法

組織	導入化学療法	対照群	症例数
SWOG/INT	CBDCA/PAC ×3	S	354
Scandinavia	CBDCA/PAC ×3?	S	90
UK/EORTC	platinum-based ×3	S	600
CHEST	CDDP/GEM ×3	S	256
Spain	CBDCA/PAC ×3	S	625
		Adj. Cx	
France	CDDP/GEM ×2 vs. ×4		520
	vs. CBDCA/PAC ×2 vs. ×4		

CBDCA：カルボプラチン，PAC：パクリタキセル，
CDDP：シスプラチン，GEM：ゲムシタビン，S：手術単独

行う群と行わない群（手術単独）との大規模な比較試験を行った[11]．この試験は目標症例数 600 例の設定であったが，症例集積約 5 年で 354 例（うち不適格例 19 例）登録されて中止に至った．観察期間中央値は 31 カ月で，生存期間中央値（MST）は化学療法群で 47 カ月，手術単独群では 40 カ月で，両群間の生存期間に統計学的な有意差はない（p=0.32）ものの，先のフランスのグループ