tions are, of course, proteins. Therefore, it is necessary to investigate human proteins to understand the pathogenesis of human diseases.

Human genome analysis has shown that there are approximately 35,000 human genes, and it is assumed that more than 100,000 proteins must be expressed in the human body. In addition, it is important to understand the three-dimensional structures of protein molecule to understand their functions. Even though it is very complicated to analyze protein molecules, we cannot avoid investigating proteins for the complete elucidation of the pathogenesis of any human diseases. Therefore, new technology that combines simplicity, high through-put and automatic analysis is required. In this context, recent advanced proteomic technologies have brought the hope of discovering novel biomarkers that can be used to detect the early stage of disease, to predict the effectiveness of therapy and to monitor disease progression. The precise prediction of both therapeutic effects and adverse reactions must lead us to individualized therapy. In this review we discuss the present status of clinical proteomics for cancer-related proteins, and especially its application in the field of lung cancer is reviewed. We believe that understanding the present concepts of proteomic analysis in the field of oncology is extremely valuable for thoracic surgeons who will develop new therapeutic strategies for malignant neoplasms. The final purpose of clinical proteomics is to improve diagnostic procedures including the early detection and exact evaluation of the biological characteristics of diseases, and to understand the molecular pathogenesis of diseases to permit novel therapeutic strategies.

Commencement of Clinical Proteomics– Two-dimensional Polyacrylamide Gel Electrophoresis (2-DE) Based Strategies

Though the word "proteome" as a technical term is new, the concepts of comprehensive protein analysis have been established from 1975. At that time, O'Farrell established high resolutional two-dimensional polyacrylamide gel electrophoresis (2-DE) for comprehensive protein analysis.[2] In this method proteins are separated using isoelectric focusing (IEF) and sequential sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Approximately 1,000 proteins can be evaluated on 2-DE gel showing isoelectric point and molecular weight.

We started proteomic analysis using the 2-DE method on surgically resected materials of solid cancers in 1989. We initially used frozen surgically resected materials, but high resolutional analysis for cancer-related proteins was not possible due to contamination with serum proteins and necrotic substances as well as normal cells such as mesenchymal cells and inflammatory cells. Until we developed a new sample preparation method using fresh surgically resected materials, it was impossible to investigate cancer-related proteins with high resolution.[3] After the establishment of this non-enzymatic sample preparation technique we investigated the histopathological magnification of each histological type of primary lung cancer using clinical materials of primary lung cancer. More than 80% of proteins detected on 2-DE gels were not specific for any histological types. However, 2-DE patterns shown by the expression of several proteins reflected the histopathological differentiation of the primary lung cancer. During this investigation we identified 13 proteins associated with histopathological features. Most well-differentiated cases show high expression rates of proteins associated with the same histopathological differentiation and very low expression rates of proteins associated with other histopathological differentiation. On the other hand, in poorly differentiated cases we recognized a relatively wide variation of expression rates in the proteins associated with histopathological differentiation. In addition, when we evaluated primary lung adenocarcinoma, cases with the typical 2-DE pattern for lung adenocarcinoma showed a favorable outcome, and on the other hand the other cases showing the atypical 2-DE patterns showed a relatively poor outcome. The classification based upon the expression of cancer-related proteins may reflect biological characteristics of the tumor as well as histological differentiation.[4]

During the investigation of histopathological differentiation-related proteins, we detected one protein with high intensity only in primary lung adenocarcinoma, TA02. This protein molecule was not expressed in either metastatic lung adenocarcinoma from the other organs or the other types of primary lung cancer except a few cases of large cell lung cancer, and in normal human tissues this molecule was distributed only in type II pneumocyte and a part of the renal tubules.[5,6] At present, we understand that TA02 is homologous with napsin A, a new type of aspartic proteinase, which is involved in the maturation of the biologically active form of surfactant apoprotein B (SpB). It is suggested that Napsin A cleaves the N-terminal peptide of SpB, resulting in a 25 kDa intermediate.[7,8] We believe that our 2-DE investigation is the first report concerning proteomic analysis using clinical samples of

primary lung cancer.

Hanash and their colleagues at the University of Michigan Medical Center constructed a database that contained protein expression data on lung cancer based upon 2-DE findings. Also, they identified histopathology-related proteins. They described the possibility of developing novel classification schemes for lung cancer and the identification of novel markers for early detection using this kind of 2-DE protein database.[9] They also investigated 93 tissue samples of lung adenocarcinoma and 10 samples of normal lung tissues, and identified 9 protein molecules with significant overexpression in lung adenocarcinoma.[10] Furthermore, they detected 46 survival-associated proteins by 2-DE. Sequentially, 33 out of these 46 proteins were identified using MS, and among these candidate proteins, phosphoglycerate kinase 1 was validated as a survival-associated protein based upon another investigation of both tissues and serum derived from the patients with non-small cell lung.[11]

Proteome Platforms Not Involving 2-DE

Though 2-DE is really one of the most powerful tools for proteomic analysis, it does have several shortcomings. It is too intricate to permit automatic analysis, and its reproducibility is sometimes poor. Also, highly abundant proteins interfere with identification of less common proteins on 2-DE gel when either total-cell lysates or tissue lysates are applied to 2-DE analysis. When either cell lysates or tissues lysates are analyzed, approximately 10^6 orders in the dynamic range seem to be required. Furthermore, it is difficult to investigate extremely acidic or basic proteins and hydrophobic proteins, e.g. membrane proteins, which are strongly associated with cellular functions as a growth factor receptor. Therefore, new technology with high through-put and wide dynamic range as well as with high sensitivity have been eagerly awaited.

After bioinformatics made remarkable progress due to the completion of the human genome project, high through-put proteomic technology rapidly developed in the last 10 years. Finally, Fenn and Tanaka received the Nobel Prize for Chemistry in 2002, and proteomic analysis using mass spectrometry (MS) became the center of attention. They developed ionization technology, which is essential for MS. Tanaka developed matrix-assisted laser desorption/ionization (MALDI), and Fenn also developed electro-spray ionization (ESI), which are essential elements in MS instrumentation.

The US Food and Drug Administration (FDA) and National Cancer Institute in the US set up a Clinical Proteomics Program in 2001. The next year they reported that a specific serum-proteomic pattern of ovarian cancer was identified compared with patients and healthy donors. They used a surface-enhanced laser desorption/ionization (SELDI) MS system, which is an affinity-based MS method using a protein chip modified with a specific chromatographic surface. The SELDI MS system is a modified MALDI MS system, and it is based upon the principle that the proteins from crude mixtures are selectively attached to specific biochemical surfaces. Some proteins as potential biomarkers candidates may show a higher binding affinity to certain surfaces than common serum proteins. Serum samples from healthy donors and disease-affected individuals are processed using this protein chip. After washing steps, matrix is added to the protein spots and each proteomic pattern is acquired. Samples can be classified into a normal group, a disease-affected patients' group and additional groups, through the application of bioinformatic algorithms. SELDI MS analysis does not absolutely rely on the actual identification of the proteins to diagnose a disease. Therefore, this system has been used to detect some biomarkers (proteomic patterns) in complex protein mixtures such as cell lysates, body fluids and serum (Fig. 1).

According to initial reports concerning ovarian cancer by Petricoin et al., the discriminatory proteomic pattern correctly identified all 50 ovarian cancer cases, including 18 stage I cases. Of the 66 cases of non-malignant diseases, 63 were recognized as not being cancer. The sensitivity was 100%, specificity 95%, and positive predictive value 94%.[12] Furthermore, they investigated early detection of prostate cancer, and suggested that pathologic states within the prostate might be reflected by changes in serum proteomic patterns in relation to serum prostate specific antigen (PSA). The proteomic pattern correctly predicted 36 out of 38 patients with prostate cancer, while 177 out of 228 patients were correctly classified as having benign conditions. For men with marginally elevated PSA levels, the specificity was 71%. They concluded that serum pattern diagnostics might be of value in deciding whether to perform a biopsy on a man with an elevated PSA level.[13] This new approach is based upon the protein patterns analysis using SELDI and may provide a more effective means to diagnose some kinds of malignant neoplasm, such as ovarian, prostate, breast, and lung cancer. Over the past three years, many investigators have reported that pattern recognition algorithms based upon SELDI MS data may be successfully used to distinguish

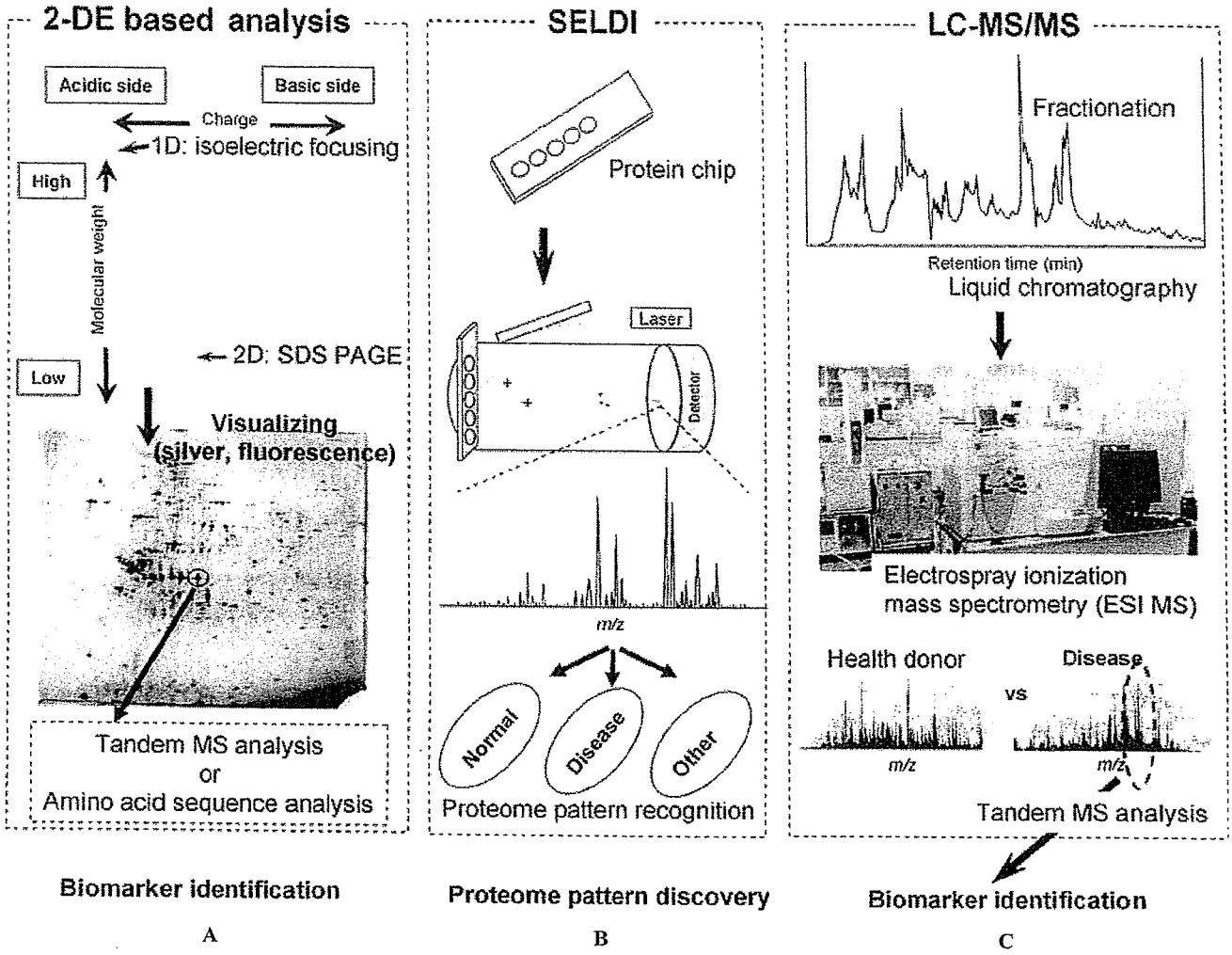

Fig. 1. Strategy for clinical proteomics.

A: Protein identification based upon two-dimensional polyacrylamide gel electrophoresis (2-DE).
A glass tube, to which the gel is cast, is used for isoelectric focusing (IEF). A sample is applied to each tube, and focused for approximately 15 hours. After the IEF gel is extruded into an equilibration buffer, sodium dodecyl sulphate (SDS) polyacrylamide gel (slab gel) is used for the second dimension. The IEF gel is sealed using agarose on the top of the slab gel, and is electrophoresed overnight. Subsequently, 2-DE proteins are visualized by either silver-staining or Comercy Blue staining.
After cutting the gel for extraction of protein, protein molecules are identified using tandem mass spectrometry (MS) or amino acid sequence analysis and bioinformatics.

B: Proteomic pattern discovery using surfaced enhanced laser desorption/ionization mass spectrometry (SELDI MS).
When the SELDI MS system is used for a diagnostic proteomics approach, samples from healthy donors and disease-affected individuals are processed using a protein chip modified with a specific chromatographic surface, and proteomic patterns are obtained as a result of the affinity to the surface of each protein chip. According to bioinformatics algorithms, the raw data are investigated to classify into either the healthy, disease, or other groups. This strategy does not rely on the actual identification of the protein molecules.

C: Protein identification using liquid chromatography-mass spectrometry (LC-MS).
This method relies upon (multidimensional) fractionation and tandem MS for protein-molecule identification. Samples derived from patients with specific diseases are compared with those derived from matched healthy donors. Attempts are made to discover unique or highly abundant proteins. Due to quantitative analysis of peptide-signal intensity and statistical analysis, it is possible to detect statistically significant differences in signal intensity, and the source protein molecule is identified using tandem MS analysis, which is performed sequentially.

between serums derived from normal donors and cancer patients.[14-17]

On the other hand, there are some reports criticizing proteomic pattern analysis using SELDI MS as not being reproducible or reliable enough for practical applications.[18,19] Finally, they concluded that the proteomic patterns that enable successful classification are biologically implausible and the methods, properly applied, do not classify the data accurately. Though commercial laboratories planned to market a test in late 2003 or early 2004, the US FDA in the US delayed starting clinical applications, because the question has not yet been resolved. We conclude that identification of a biomarker molecule is necessary when using serum biomarkers clinically, even though combination diagnosis with several kinds of biomarkers is undertaken.

Proteomic Analysis of Lung Cancer Using MS

There are few reports concerning proteomic analysis using MS for the investigation of clinical lung cancer materials. Yanagisawa et al. investigated proteomic patterns of non-small cell lung cancer using MALDI-time of flight MS (MALDI-TOF MS). They reported that class-prediction models completely classify histology, distinguish primary tumors from metastatic lesions from other organs to the lung, and classified nodal involvement with 85% accuracy. Also, they obtained a proteomic pattern comprised of 15 distinct MS peaks that distinguish between patients with poor prognosis and good prognosis.[20] However, they went no further than recognizing the proteome pattern. Therefore, the molecules related to specific events were not identified in this study.

Tyan investigated pleural effusion fluid derived from lung adenocarcinoma using two-dimensional liquid chromatography (LC) tandem MS, and it was reported that 124 proteins were identified. Based upon previous reports, it was concluded that 69 proteins among these proteins originated from plasma and that another 13 proteins were synthesized in the lung. Finally, a protein database concerning human pleural effusion will provide potential protein diagnostic biomarkers to be examined in further investigations.[21]

We also continue to make efforts to explore biomarkers related to lung cancer using LC-MS. Recently, we established a high-throughput comprehensive protein profiling system comprising a fully automated on-line microflow LC/tandem MS system for clinical sample utility. In this system quantitative evaluation of signal intensity analyzes statistically significant differences between two groups, for instance groups of healthy donors and cancer patients. Furthermore, automatic operation enabled the completion of a single run of entire LC-MS/MS analysis within 11 hours. Investigation of the data extracted from the protein identification datasets of both groups could allow identification of candidate proteins of disease-specific biomarkers. We applied this high throughput micro LC-MS/MS protein profiling system to surgically resected tissues and plasma derived from patients with primary lung adenocarcinoma, and attempted to identify the specific protein-molecules showing statistically significant differences in protein-expression levels. Firstly, we applied this protein profiling system to the investigation of the proteins associated with lymph node metastasis compared with expression profiles of two groups. One group consisted of cases without lymph node involvement, and the other group consisted of cases less than 3 cm in the largest dimension with lymph node involvement. The profiles were accumulated for each group using our originally developed profile alignment program. The statistical selection was done by Student's t test using a p-value of less than 0.005 as a cut-off value. The number of selective data points was 5,889. We have to note that the number of data points is not identical to the number of proteins. Generally, one protein produces several kinds of peptide molecular ions, and each signal is expected to correspond to a single LC-MS signal. Among 5,889 significant points, 2,753 associated with protein identification information were derived from MS/MS data by the MASCOT™ protein identification software. Finally, we identified more than 500 protein molecules as potential biomarkers associated with lymph node involvement. However, a validation process is needed for clinical application of these results.

We also started a project to mine novel biomarkers for the early detection of primary lung adenocarcinoma. We believe that such protein may leak from cancerous tissue but their concentration may be less than 100 femto mol. The lower limitation of the detectable range in LC-MS system is the same order as the concentration of tissue leakage proteins. Plasma proteins consist of a large amount of classical proteins including albumin, globulin, several kinds of complement and fibrinogen, and a very small amount of tissue leakage proteins and interleukins. We used serum albumin- and Immunoglobulin-depleted samples in plasma proteome analysis, and succeeded in identifying more than 100 proteins.[22] However, at present it is difficult to reliably detect a very small

amount of protein such as tissue-leakage proteins derived from malignant solid tumor. We believe that a multi-dimensional analysis system is needed before MS analysis for plasma biomarker discovery, which is the primary aim in clinical plasma proteome projects.

Conclusion

Though recent advances in proteomic analysis are conspicuous, nevertheless current research has not yet achieved the final clinical goal of producing specific biomarkers for the early detection and discovery of new molecular targets for individualized therapy. The target molecules to be detected in proteomic investigations, must be sufficiently abundant to be clinically useful. It is necessary to further improve our techniques concerning sensitivity and wide dynamic range sufficiently to analyze human plasma proteins. We believe that the remaining problems will be resolved within a few years, and that a fundamental revolution in both early detection and therapeutic strategy will occur in the near future.

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

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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

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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

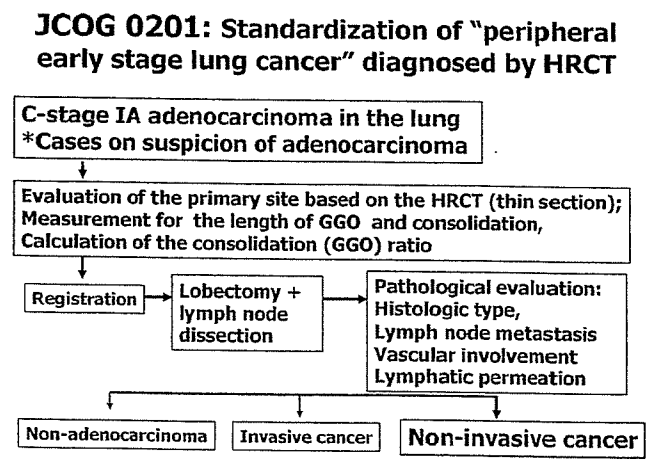

FIGURE 1. Schema for JCOG 0201 trial.

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.

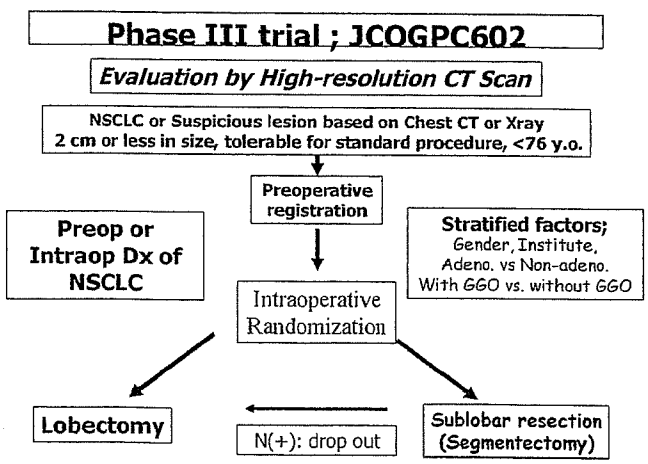

FIGURE 2. Schema for JCOGPC 602 trial.

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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REVIEW ARTICLE

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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]

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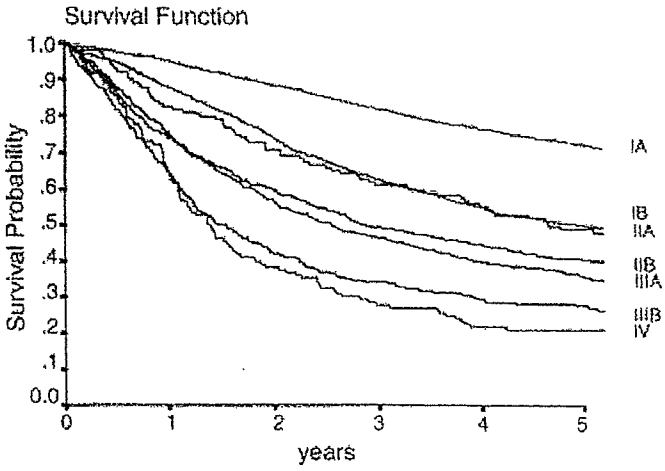

Fig. 1. Survival curves according to clinical (c)-stage. The 5-year survival rates according to c-stage were as follows: 72.1% for IA (n = 2423), 49.9% for IB (n = 1542), 48.7% for IIA (n = 150), 40.6% for IIB (n = 746), 35.8% for IIIA (n = 1270), 28.0% for IIIB (n = 366), and 20.8% for IV (n = 147). 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 ≦ 1 cm	1 < T ≦ 5 cm	1.5 < T ≦ 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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7196 General Poster Session (Board #U5), Sun, 8:00 AM - 12:00 PM

Prognostic value of blood levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in advanced non-small cell lung cancer (NSCLC) patients. *V. Alberola, C. Camps, R. Sirera, L. Llobat, A. Blasco, M. J. Safont, J. Garde, M. Taron, J. J. Sanchez, R. Rosell; Hospital Arnau de Vilanova, Valencia, Spain; Hospital General de Valencia, Valencia, Spain; Hospital Germans Trias i Pujol, Badalona, Spain; Universidad Autonoma de Madrid, Madrid, Spain*

Background: VEGF and bFGF are among the most important angiogenic factors. We have explored these angiogenesis mediators in plasma and its prognostic significance in advanced NSCLC. **Methods:** Were enrolled 451 patients with advanced NSCLC, stages IIIB and IV and treated with cisplatin and docetaxel. Blood was collected before chemotherapy. Plasma VEGF and bFGF levels were assessed by commercial ELISA (sensitivity 5 pg/ml). In parallel plasma from 32 age and gender-matched controls was used. **Results:** Median age was 61 years (35–82) and 84% were males. 99% had performance status 0–1. 84% were in stage IV and 16% in stage IIIB. The histological subtypes were: 32% squamous cell carcinoma, 50% adenocarcinoma, 14% anaplastic large cell, and 4% undifferentiated. 41% of the patients received second line chemotherapy. 1% achieved complete response (CR), 36% partial response (PR), 35% had stable disease (SD) and 28% progressive disease (PD). Patient's median plasma levels of VEGF (20 pg/ml, [6–203]) differ significantly ($p = 0.04$) from controls (14 pg/ml, [7–53]), but in contrast bFGF levels were not different, 14 pg/ml [5–960] vs 10 pg/ml [6–278] respectively. There were not differences in patients according to histology, site of metastasis and ECOG; however we could observe a tendency with stage for both factors: bFGF 9 pg/ml [5–24] in stage IIIB vs 15 pg/ml [6–960], $p = 0.071$ and VEGF 17 pg/ml [6–145] in IIIB vs 21 pg/ml [6–203] in IV, $p = 0.086$. It could not be observed any differences in response to therapy for both angiogenic factors; CR+PR patients presented median VEGF of 18 pg/ml [6–71] and bFGF 11 pg/ml [6–960] vs 20 pg/ml of VEGF [6–203] and 15 pg/ml of bFGF [5–395] in the SD+PD group. In the multivariate analysis we could not find that VEGF and bFGF plasma levels were predictors for time to progression (TTP) and overall survival (OS). **Conclusions:** VEGF but not bFGF levels in patients are significantly higher in patients than in controls. In our cohort of patients with advanced NSCLC we have not found any relationship between serum VEGF and bFGF levels with stage, histology, response, site of metastasis, TTP and OS.

7197 General Poster Session (Board #U6), Sun, 8:00 AM - 12:00 PM

The influence of tumor size, histological differentiation and smoking history in patients with completely resected stage I adenocarcinoma of the lung. *M. Tsuboi, H. Kato, Y. Ichinose, M. Ohta, E. Hata, N. Tsubota, H. Tada, H. Wada, N. Hamajima, M. Ohta, the Japan Lung Cancer Research Group on Postsurgic; Tokyo Medical University, Tokyo, Japan; National Kyushu Cancer Center, Fukuoka, Japan; National Okinawa Hospital, Okinawa, Japan; Mitsui Memorial Hospital, Tokyo, Japan; Hyogo Medical Center, Akashi, Japan; Osaka City General Hospital, Osaka, Japan; Kyoto University, Kyoto, Japan; Nagoya University, Nagoya, Japan*

Background: To test the hypothesis that patients with completely resected p-stage I adenocarcinoma [Ad.] of the lung contain a favorable subgroup of patients with well differentiated histology and tumor 2.0 cm or less in greatest dimension, we analyzed the results of the JLCRG trial (a randomized prospective trial of adjuvant chemotherapy with Uracil-Tegaful for stage I adenocarcinoma of the lung) by tumor size, smoking history, degree of histological differentiation and more. **Methods:** Patients were randomized to receive either oral uracil-tegaful (250 mg of tegaful /m^2/day) for 2 years postoperatively or no adjuvant treatment. Multivariate analyses and interactions with the Cox proportional-hazards model were used to estimate the simultaneous effects of prognostic factors on survival. **Results:** The 5-year survival rate of the 412 patients with tumor 2cm or less in size was 89.8% (95% confidence interval [CI]: 86.8 to 92.8) versus 84.4% (95% CI: 81.3–87.4) for the 569 patients with tumor more than 2cm in size (median follow-up 72 months, $p = 0.002$). Although univariate analysis demonstrated improved survival for the patients with no smoking history and female gender, the selected covariates by multivariate analysis were as follows: age (hazard ratio [HR] for patients aged 70 years or more, 2.25; 95% CI: 1.58 to 3.14, $p < 0.0001$), tumor size (HR for more than 2cm in size, 1.55; 95% CI: 1.10 to 2.21, $p = 0.012$), histological differentiation (HR for moderate and poor differentiation, 1.75, 95% CI: 1.25 to 2.47, $p = 0.001$), and treatment group (HR for the uracil-tegaful group, 0.68; 95% CI: 0.49 to 0.94, $p = 0.02$). For these prognostic factors, there was only one significant interaction between tumor size and the adjuvant treatment. **Conclusions:** 1) Patients with completely resected stage I Ad. of the lung contain a favorable subgroup of patients with aged less than 70 years, well differentiated histology, and a maximum tumor dimension of 2.0 cm or less. 2) Adjuvant chemotherapy with oral uracil-tegaful should also be considered for stage I Ad. patients more than 2 cm in tumor size. 3) 2cm in tumor size might be a good benchmark candidate of the description of T factor to facilitate treatment strategies and revisions of the TNM staging system.

7198 General Poster Session (Board #U7), Sun, 8:00 AM - 12:00 PM

Elevated osteopontin (OPN) plasma levels are highly prognostic in advanced non-small cell lung cancer (NSCLC): Analysis of SWOG S0003. *P. C. Mack, M. W. Redman, K. Chansky, S. K. Williamson, N. Farneth, P. N. Lara Jr, Q. Le, P. H. Gumerlock, J. J. Crowley, D. R. Gandara; UC Davis Cancer Center, Sacramento, CA; Southwest Oncology Group Statistical Center, Seattle, WA; SWOG Statistical Center, Seattle, WA; University of Kansas Medical Center, Kansas City, KS; Stanford University, Stanford, CA*

Background: OPN is a secreted glycoprotein with a diverse array of functions, including induction of uPA & increased cell migration. OPN has been shown to be elevated in a number of tumor types, & its downregulation reduces tumorigenicity & metastasis in tumor models. High levels have also been associated with tumor hypoxia/angiogenesis, as are vascular endothelial growth factor (VEGF) & plasminogen activator inhibitor (PAI-1). We hypothesized that secreted levels of these biomarkers would correlate with clinical outcome after treatment. **Methods:** Plasma concentrations of OPN, VEGF & PAI-1 were measured by ELISA in 160 NSCLC patients enrolled on the Southwest Oncology Group (SWOG) trial S0003 (paclitaxel/carboplatin ± the hypoxic cytotoxin tirapazamine). Post-treatment plasma samples were available in 56 patients. **Results:** Baseline OPN plasma levels correlated significantly with patient overall survival (OS). High interpatient variability was observed, with levels ranging from undetectable to 2560 ng/ml, (median: 606.5 ng/ml). When dichotomized, median OS was 11 months for patients below median OPN levels & 7 months for those above ($p = 0.004$). Survival decreases with increasing OPN concentration. Furthermore, OPN levels correlated with response rate (RR) (median responders: 497; median non-responders: 698 ng/ml. Wilcoxon rank-sum $p = 0.03$). No association between baseline levels of either VEGF or PAI-1 with RR or OS was observed. However, plasma levels of both PAI-1 & VEGF were significantly inter-related & trended together ($p < 0.0001$), & both decreased significantly after treatment ($p = 0.0004$ & 0.04, respectively). Median decrease: OPN: 17%, PAI: 44%, VEGF: 42%. No significant differences were observed between study arms, suggesting that OPN is prognostic in NSCLC, but not predictive for response to tirapazamine. **Conclusions:** 1) There is a great need for development of tumor biomarkers which can be serially assessed pre- & post-therapy. 2) High OPN plasma levels were significantly associated with reduced RR & OS for patients on this trial. OPN is a strong candidate for inclusion in a panel of prognostic (& perhaps predictive) markers for NSCLC. Supported by the Hope Foundation & RO1-CA107228.

7199 General Poster Session (Board #U8), Sun, 8:00 AM - 12:00 PM

The relationship between RASSF1A aberrant methylation and survival in small sized lung adenocarcinoma. *K. Miyajima, T. Ohira, J. Usuda, H. Saji, M. Tsuboi, T. Hirano, H. Kato, M. Suzuki, S. Toyooka, A. F. Gazdar; Tokyo Medical University, Tokyo, Japan; University of Texas Southwestern Medical Center, Dallas, TX*

Background: Aberrant methylation of CpG islands in promoter regions of tumor cells is one of the major mechanisms for silencing of tumor suppressor genes. The RAS association domain family 1A (RASSF1A) gene was isolated from the 3p21.3 region homozygously deleted in lung cancer cell lines, and it was shown to be inactivated by hypermethylation of the promoter region in lung cancers. In this study, we investigated the clinicopathological significances of RASSF1A methylation in the development and/or progression of small-sized (less than 2.0cm) lung adenocarcinoma. It is important to identify a marker for high-risk early stage patients who should benefit from new investigational adjuvant therapies. **Methods:** Surgically resected specimens from 77 cases of small-sized primary lung adenocarcinoma. We determined the frequency of aberrant promoter methylation of the RASSF1A genes in small-sized adenocarcinoma. Aberrant promoter methylation was examined using methylation-specific PCR (MSP). **Results:** Twenty-five of 77 (32.5%) tumors showed RASSF1A methylation. RASSF1A methylation was dominantly detected in smoker ($P < 0.03$). There was no significant correlation of RASSF1A methylation with gender, age, T stage, N stage and pathological stage. RASSF1A methylation correlated with adverse survival by univariate analysis ($P < 0.005$) as well as multivariate analysis ($P = 0.0062$; RR 4.251; 95% C.I., 1.507–11.993). Furthermore, RASSF1A promoter hypermethylation in resected stage I small-sized lung adenocarcinoma was associated with impaired patient survival ($P < 0.01$). **Conclusions:** Aberrant promoter methylation of the RASSF1A was present in 25 of 77 (32.5%) of small-sized lung adenocarcinoma by MSP assay. These results indicated that epigenetic inactivation of RASSF1A plays an important role in the progression of small-sized lung adenocarcinoma, and that RASSF1A hypermethylation appears to be a useful molecular marker for the prognosis of patients with small-sized and stage I lung adenocarcinoma. RASSF1A is a potential tumor suppressor gene that undergoes epigenetic inactivation in lung adenocarcinoma through hypermethylation of its promoter region. RASSF1A methylation was significantly related to unfavorable prognosis in small-sized lung adenocarcinoma.

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A randomized phase III trial of adjuvant treatment for resected non-small cell lung cancer in Japan

Yukito Ichinose

Abstract Two randomized phase III trials [uracil–tegafur (UFT) and bestatin trials] of adjuvant treatment for resected stage I non-small cell lung cancer (NSCLC) have recently been completed in Japan. The Japan Lung Cancer Research Group on Postsurgical Adjuvant Chemotherapy conducted a phase III trial in which 999 patients with completely resected stage I adenocarcinoma were assigned to receive either oral UFT [tegafur, 250 mg/(m^2 day)] for 2 years or no treatment (January 1994–March 1997). At a median follow-up time of 73 months, the overall survival in the UFT group was significantly higher than that in the control group ($P = 0.035$). Grade 3 toxic effects occurred in 10 of the 482 patients (2%) who received UFT. Since 1985 when UFT became available in Japan, a total of 6 phase III trials comparing adjuvant chemotherapy using UFT with observation alone, including the above trial, have been conducted. A meta-analysis of these six trials reconfirmed that UFT had a beneficial effect in patients with completely resected stage I NSCLC. A phase III trial comparing UFT with platinum-based chemotherapy in patients with completely resected NSCLC with pathological stage IB through IIIA disease is also now under consideration. Bestatin is a potent aminopeptidase inhibitor that has both immunostimulant and antitumor activities. A prospective randomized, double blind, placebo-controlled trial in patients with completely resected stage I squamous cell carcinoma was conducted. A total of 402 patients were randomly assigned to receive either oral bestatin 30 mg/day or a placebo daily for 2 years (July 1992–March 1995). At the median follow-up time of 76 months, the overall survival in the bestatin group was significantly higher than that of the placebo control group ($P = 0.033$). The 5 year overall survival was 81% in the bestatin group and 74% in the placebo group. Few adverse events were observed in either group. These results, however, require further confirmation in other phase III trials.

Keywords Non-small cell lung cancer · Postoperative adjuvant treatment · UFT · Bestatin · Stage I

Introduction

Two randomized phase III trials [uracil–tegafur (UFT) and bestatin trials] of adjuvant treatment for resected stage I non-small cell lung cancer (NSCLC) have recently been completed in Japan.

UFT trial

UFT is an oral anticancer agent composed of tegafur and uracil at a molar ratio of 1:4, which has good absorption from the small intestine [6]. Tegafur is gradually converted to 5-fluorouracil via the metabolism of liver enzyme P450. Uracil enhances the serum 5-fluorouracil concentration by the competitive inhibition of

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dihydropyrimidine dehydrogenase, the enzyme responsible for 5-fluorouracil catabolism [11]. Administration of oral UFT reportedly generates a higher maximum plasma level of 5-fluorouracil than the protracted intravenous injection of 5-fluorouracil given in a dose equimolar to the tegafur in UFT [9].

The West Japan Study Group for Lung Cancer Surgery reported that postoperative adjuvant treatment with UFT in patients with completely resected stage I–III disease prolonged survival significantly compared with observation alone [33]. The 5 year survival rate was 64% in the UFT group and 49% in the control group ($P = 0.02$). In a subgroup analysis, no statistically significant difference between the two groups was observed in the overall survival of patients with squamous cell carcinoma ($P = 0.24$). In contrast, patients with adenocarcinoma in the UFT group had a significantly better survival than those in the control group ($P = 0.009$) [21]. Of note, most patients with adenocarcinoma had stage I disease. Those results prompted us to conduct a prospective randomized trial of UFT as a postoperative adjuvant treatment for patients whose stage I adenocarcinoma was completely resected.

Between January 1994 and March 1997, 999 patients who had undergone a complete resection of a pathologically documented stage I (T1–2, N0, M0) [18] adenocarcinoma were enrolled in the trial. Patients were randomly assigned to receive either no treatment ($n = 501$) or UFT ($n = 498$), respectively. However, 13 patients in the control group and 7 patients in the UFT group were found to be ineligible. Therefore, the number of all eligible patients was 488 in the control group and 491 in the UFT group. The patients assigned to the control group were observed with no further treatment after surgery. In the UFT group, UFT (tegafur 250 mg/m^2 of body-surface area) in the form of a 100 mg capsule (100 mg tegafur and 224 mg uracil) was given orally in two separate doses, before meals, daily for 2 years, starting 4 weeks after surgery. The clinical characteristics of those eligible patients are listed in Table 1. There were no statistically significant differences in the baseline characteristics of the patients. All but one patient in each group underwent lobectomy.

Of the 498 patients randomized to the UFT group, 482 patients received oral UFT. Table 2 lists the incidence of UFT-related adverse reactions. Few severe adverse reactions were associated with UFT administration. There was no grade 4 adverse reaction. In total, 10 (2%) of 482 patients developed a grade three adverse reaction.

The median follow-up for the surviving patients was 72 months in the UFT group and 73 months in the control group. The 5 year survival rate was 88% [95% confidence interval (CI): 85–91%] in the UFT group and 85% (95% CI: 82–89%) in the control group (Fig. 1).

Table 1 Patient characteristics in the UFT study

Characteristic	UFT ($n = 491$)	Control ($n = 488$)
Age		
Mean (years)	62	62
Range (years)	45–75	45–75
<65	274	275
≥65	217	213
Female sex	253	249
ECOG performance status[a]		
0	376	369
1	105	113
2	10	6
Pathological T status		
T1	362	354
T2	129	134
Pleural invasion[b]		
0	340	346
1	120	114
2	29	28
Unknown	2	0
Tumor size		
≤2 cm	208	204
>2 to ≤3 cm	174	170
>3 cm	109	114
Location of the tumor		
Right upper lobe	182	189
Right middle lobe	41	34
Right lower lobe	102	87
Right lobes	2	2
Left upper lobe	107	114
Left lower lobe	54	60
Left lobes	3	2
Operation modality		
Lobectomy	490	487
Pneumonectomy	1	1

[a] ECOG Eastern Cooperative Oncology Group. Higher performance status numbers indicate greater impairment

[b] *0* a tumor with no pleural involvement or a tumor that reaches the visceral pleura, but does not extend beyond the elastic layer; *1* a tumor that extends beyond the elastic layer of the visceral pleura, but is not exposed on the pleural surface; and *2* a tumor that is exposed on the pleural surface, but does not involve the parietal pleura

The predetermined covariates were age (<65 vs ≥65 years), sex (male vs female), performance status (0 vs 1 + 2), T status (T1 vs T2), and treatment group. The covariates were selected according to multivariate analysis using a stepwise procedure under the condition that the *P*-value was less than 0.05. The selected covariates were as follow: age (hazard ratio = 2.02, 95% CI: 1.46–2.80; $P < 0.001$), T status (hazard

Table 2 Adverse reactions to UFT ($n = 482$)

Adverse reaction	Grade of toxicity[a]			
	1	2	3	4
Percent of patients				
Leukopenia	2	1	0	0
Thrombocytopenia	<1	0	0	0
Anemia	1	<1	0	0
Increase in bilirubin	1	<1	0	0
Increase in AST	6	2	<1	0
Increase in ALT	6	2	0	0
Increase in ALP	2	<1	0	0
Anorexia	9	8	1	0
Nausea/vomiting	10	3	1	0
Diarrhea	2	1	<1	0
Alopecia	<1	0	0	0

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[a] Toxicity was graded according to criteria of the Japan Society of Clinical Oncology. Grades range from 1 to 4, with a higher grade indicating a more severe reaction

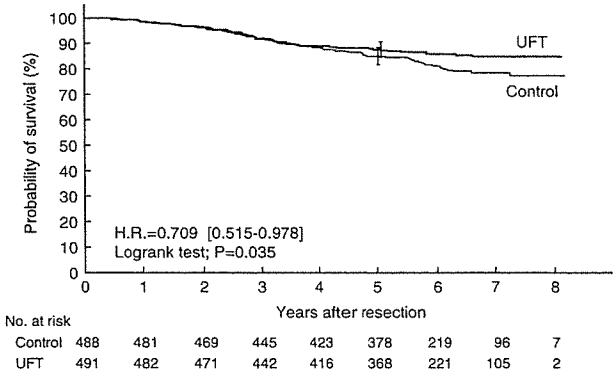

Fig. 1 Overall survival of all eligible patients ($n = 979$). *Error bars* represent the 95% confidence intervals. Reprinted with permission from the New England Journal of Medicine [15]. Copyright© 2004 Massachusetts Medical Society. All rights reserved

ratio = 1.95, 95% CI: 1.41–2.69; $P < 0.001$), sex (hazard ratio = 0.66, 95% CI: 0.48–0.91; $P = 0.01$), and treatment group (hazard ratio = 0.72, 95% CI: 0.53–1.00; $P = 0.05$).

The interaction between four prognostic factors and the treatment was then evaluated (Fig. 2). Since the T status is mainly classified by the maximum diameter of the primary tumor, we added the tumor size to the analysis. As shown in Fig. 2, a significant interaction between either T status or the tumor size with the treatment was observed.

The patients with T2 disease in the UFT group had a significantly better survival than those in the control group, while there was no survival difference between the UFT and the control group in the patients with T1 disease. The 5 year survival rate of patients with T2 disease

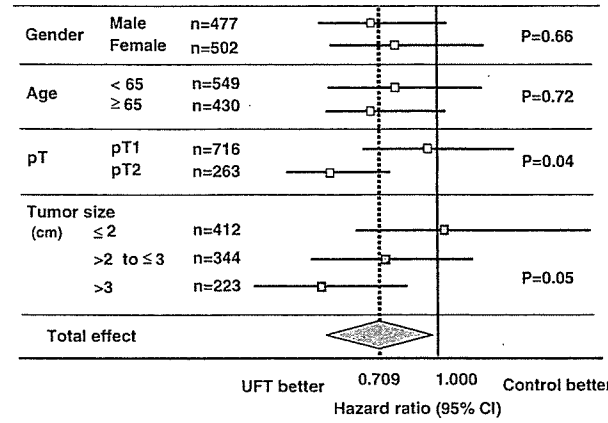

Fig. 2 Hazard ratios for death in patients in the UFT group compared with the control group, according to four prognostic factors. Each square represents the estimated treatment effect, and the horizontal lines represent the 95% confidence intervals. The diamond corresponds to the 95% confidence intervals for the entire group of patients. The *P*-value for the tumor size is for the comparison of patients who had tumors that were 2 cm or less in diameter with patents who had tumors that were more than 3 cm. Reprinted with permission from the New England Journal of Medicine [15]. Copyright© 2004 Massachusetts Medical Society. All rights reserved

was 85% (95% CI: 79–91%) in the UFT group and 74% (95% CI: 68–81%) in the control group (Fig. 3). The overall survival between the two groups was statistically significantly different ($P = 0.005$ by the log-rank test). The 5 year survival rate of patients with T1 disease was 89% in the UFT group and 90% in the control group ($P = 0.87$).

Until now, six randomized trials [4, 12, 20, 28, 33], including the present trial, comparing surgery alone with postoperative adjuvant treatment with UFT, have been conducted. Among them, three trials demonstrated a

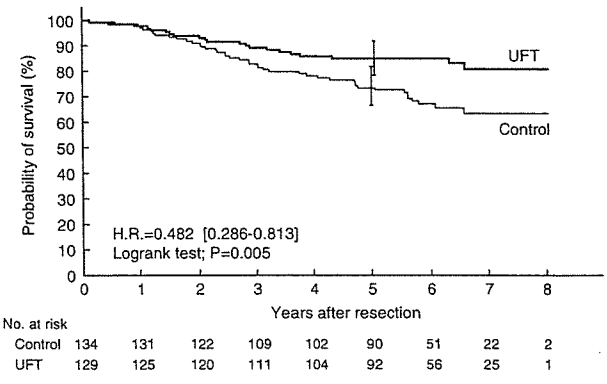

Fig. 3 Overall survival of all eligible patients with T2 disease ($n = 263$). *Error bars* represent the 95% confidence intervals. Reprinted with permission from the New England Journal of Medicine [15]. Copyright© 2004 Massachusetts Medical Society. All rights reserved

survival benefit for UFT [28, 33]. In addition, the results of a meta-analysis of those six trials demonstrated that adjuvant chemotherapy with UFT improved the overall survival (hazard ratio = 0.77, 95% CI: 0.63–0.94; $P = 0.01$) [8]. Whether the patients with stage II or III have a survival benefit from UFT treatment or whether the 1 year treatment is equivalent to the 2 year treatment remains unclear. However, patients with completely resected stage I disease, especially T2N0 adenocarcinoma, might be recommended to receive postoperative adjuvant chemotherapy with UFT based on the results of the present study.

Bestatin trial

Bestatin [(−)-*N*-[(2*S*,3*R*)-3-amino-2-hydroxy-4-phenyl-butyryl]-L-leucine] is an immunostimulator isolated from a culture filtrate of *Streptomyces olivoreticuli* that enhances the concanavalin A-induced activation of lymphocytes [14, 31]. This molecule inhibits the aminopeptidase N, aminopeptidase B, and leucine aminopeptidase of mammalian cells [16, 19]. Aminopeptidase N is identical to the myeloid differentiation antigen CD13 [1, 17] and is now considered to be a ubiquitous cell surface zinc aminopeptidase involved in the down-regulation of regulatory peptide signals [3, 24, 25, 27, 30]. Aminopeptidase N/CD13 is also reported to be involved in both tumor cell invasion [7, 26] and tumor angiogenesis [2, 23]. In addition, the expression of aminopeptidase N/CD13 in a tumor tissue is found to be an adverse prognostic factor in resected patients with lymph node-positive colon cancer [5].

In clinical studies, bestatin has demonstrated a prolongation of survival in adult acute nonlymphocytic leukemia in combination with chemotherapy [22, 32] and also an immunomodulatory effect in patients with lymphoma following autologous bone marrow transplantation [13]. Although a single institutional randomized clinical trial with bestatin as a postoperative adjuvant treatment in subjects with resected non-small cell lung cancer did not show any conclusive results, due to the small sample size, subset analysis indicated that bestatin could prolong survival in patients with completely resected pathological stage I squamous cell carcinoma [35]. This result prompted us to conduct a prospective, multi-center, randomized, double blind, placebo-controlled trial of bestatin as a postoperative adjuvant treatment for pathological stage I patients whose squamous cell carcinoma was completely resected.

From July 1992 through March 1995, 402 patients who had undergone a complete resection of pathologically documented stage I (T1–2N0M0) squamous cell carcinoma were enrolled in the trial. Two patients rescinded their informed consent before the start of treatment. The characteristics of patients who were randomly assigned to receive bestatin ($n = 202$) and placebo ($n = 198$) are shown in Table 3. There were no significant differences in the baseline characteristics of the patients. One capsule of either bestatin 30 mg or a placebo (vehicle without active drug) was orally administered after breakfast every day for 2 years postoperatively. The oral administration started within 1 week after the randomization.

There were few severe adverse reactions related to treatment and no significant difference in the incidence

Table 3 Patient characteristics in the bestatin trial

Characteristic	Bestatin ($n = 202$)	Placebo ($n = 198$)	P-value[a]
Age			
Median (years)	65	66	0.1828
Range (years)	41–76	45–75	
<65	83	66	0.1087
≥ 65	119	132	
Male sex	181	180	0.6600
ECOG performance status[b]			0.3906
0	117	125	
1	81	65	
2	4	8	
Tumor status			0.9655
Tis[c]	0	2	
1	99	95	
2	103	100	
3	0	1	
Location of the tumor			0.1724
Right upper lobe	44	64	
Right middle or lower lobe	53	51	
Right upper and middle lobe[d]	1	1	
Right upper and lower lobe[d]	0	2	
Left upper lobe	60	45	
Left lower lobe	42	34	
Left upper and lower lobe[d]	2	1	
Operation modality			0.4029
Lobectomy	197	189	
Pneumonectomy	5	8	
Segmentectomy	0	1	
Blood transfusion			0.9697
Done	36	35	
Not done	166	163	

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[a] All statistical tests were two-sided

[b] ECOG denotes Eastern Cooperative Oncology Group. Higher performance status numbers indicate greater impairment

[c] Carcinoma in situ

[d] The tumor was located between the lobes

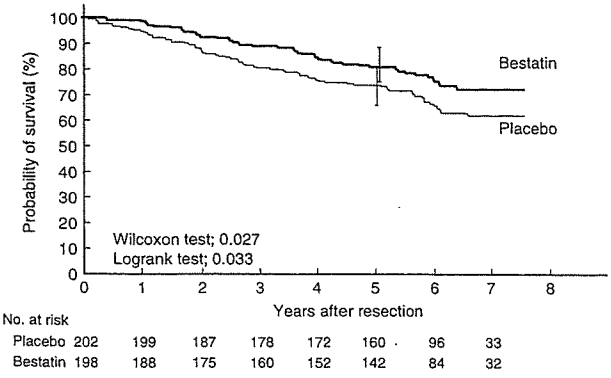

Fig. 4 Overall survival of all eligible patients ($n = 400$). *Error bars* represent the 95% confidence intervals. Reprinted with permission from Oxford University Press [10]

of adverse reactions was observed between the groups, with the exception of anorexia. In the bestatin group, 18 patients had grade 1 anorexia, 6 had grade 2, and 5 had grade 3, while in the placebo group, 8 had grade 1, 1 had grade 2, and 4 had grade 3 anorexia. The incidence of anorexia of any grade was 15% (29/196) in the bestatin group and 7% (13/189) in the placebo group ($P = 0.0127$).

The median duration of follow-up for the surviving patients was 76 months (range, 58–92 months). As shown in Fig. 4, the 5 year survival rate was 81% (95% CI: 76–86%) in the bestatin group and 74% (95% CI: 68–80%) in the placebo group. The difference in survival was significant ($P = 0.033$ by the log-rank test, $P = 0.027$ by the Wilcoxon test: without a covariate adjustment).

In the multivariate analysis, the predetermined covariates were age, sex, ECOG performance status, T status, and blood transfusion, which are generally reported to affect prognosis [29, 34]. The covariates were selected according to the forward stepwise procedure ($P < 0.1$).

The selected covariates [*P*-value, comparison, relative risk (95% CI)] were as follows: age [$P = 0.003$, <65 vs ≥65 years, 1.92 (1.24–9.95)], performance status [$P = 0.010$, 0 vs 1 and 2, 1.63 (1.13–2.35)], blood transfusion [$P = 0.021$, no vs yes, 1.63 (1.08–2.47)], and sex [$P = 0.050$, female vs male, 2.28 (1.00–5.19)]. After adjustment, the result for the treatment group comparison [$P = 0.034$, bestatin vs placebo, 1.49 (1.03–2.16)] was almost the same as without any adjustment ($P = 0.033$).

In conclusion, the oral administration of bestatin as a postoperative adjuvant treatment was found to significantly prolong the survival of patients with completely resected stage I squamous cell carcinoma without any significant adverse events. However, to confirm the present conclusions, another phase III trial should be conducted.

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