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STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

LUNG CANCER — PART IV
NON-SMALL-CELL LUNG CANCER-EPIDEMIOLOGY, TUMOR MARKERS, DIAGNOSIS, STAGING, AND PROGNOSIS

EPIDEMIOLOGY 1129
Gender Variations in the Incidence of NSCLC 1129
Age Factors in the Incidence of NSCLC 1131
Stage at Diagnosis 1131
Survival 1131
NSCLC Incidence by Histologic Type 1131
TUMOR MARKERS 1133
Hereditary Factors 1133
Other Biomarkers 1135
DIAGNOSIS AND SCREENING 1135
Noninvasive Imaging 1135
Electron beam tomography (EBT) 1135
Positron emission tomography (PET) 1135
In vitro Approaches 1135
Cell-associated transferrin receptor (TfR) 1135
Cyfra 21-1 1135
DR-70 1136
Gastrin-releasing protein receptor (GRPr) 1136
Heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 1136
Homeobox (HOX) genes 1141
MAb 28K29 1142
Micrometastases detection 1142
Microsatellite alterations 1142
Telomerase 1142
STAGING 1143
Revisions in the International System for Staging Lung Cancer 1143
Noninvasive Imaging 1143
CT staging 1143
FDG PET imaging 1143
MR scanning 1144
Surgical Staging 1144
Mediastinotomy/mediastinoscopy 1144
Thoracoscopy and video-assisted thoracoscopy (VATS) 1144
Intra-operative sentinel lymph node mapping 1144
Molecular Substaging 1145
PROGNOSIS 1146
Serum Markers 1146
Tissue Pathology 1147
Angiogenesis evaluation 1147
Genetic Tests 1147
FDG PET Imaging 1148

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NON-SMALL-CELL LUNG CANCER-EPIDEMIOLOGY, TUMOR MARKERS, DIAGNOSIS, STAGING, AND PROGNOSIS

This issue, one of a 6-part review of lung cancer, focuses on the epidemiology, diagnosis, and prognosis of non-small-cell lung cancer (nsclc). Consisting of several histologic types, and comprising 80% of all lung cancer in the USA, it represents the bulk of primary lung cancer cases both in the USA and worldwide with an annual global incidence of about 600,000 cases.

EPIDEMIOLOGY

Non-small-cell lung cancer is the leading lung cancer type worldwide, comprising three major subtypes, squamous cell carcinoma (SCC), adenocarcinoma, and large-cell cancer (Exhibit 1). Because of the major influence of tobacco in the development of lung cancer, incidence of the various subtypes of this malignancy differs by geographic location, and is predicated on tobacco exposure. Generally, however, and specifically in males, global incidence of lung cancer subtypes is similar because smoking among males is prevalent worldwide.

Gender Variations in the Incidence of NSCLC

It is very difficult to establish a gender-based influence in the incidence of nsclc. However, population studies have found that women are at substantially increased risk of smoking-related lung cancer. In the early 1990s, smoking-related lung cancer was almost exclusively diagnosed

in males. It is believed this phenomenon was attributable to the fact that, historically, most men smoked while it was considered unseemly for women to do so. But, over the past 50 years, as more and more women began to smoke, rates among women climbed while those among men stabilized. Currently, the gap between lung cancer incidence rates between men and women has been narrowing.

An example of this trend comes from the Czech Republic that has one of the highest lung cancer mortality rates in Europe. Standardized mortality rates from lung cancer in Czech men increased from 22.3/100,000 in 1950 to 79.8/100,000 in 1986, and decreased to 69.7/100,000 in 1995. Among females rates increased from 4.3/100,000 in 1950, to 8.5/100,000 in 1986, and 11.6/100,000 in 1995; a decrease to 68.0/100,000 for men, and an increase to 16.1/100,000 for women has been predicted by the end of the next decade.

During this period, there was an increase in the cigarette consumption in the Czech Republic from 1,731 cigarettes per 1 inhabitant ≥15 years-of-age in 1953, to 2,552 cigarettes in 1978, and a subsequent decrease to 2,279 cigarettes in 1989. Although some decreases in the age-specific lung cancer mortality rates were observed in young adult men, and, for the next decade, similar trends have been predicted for middle-aged men, the trend has been upward in all age groups among females. There was a downward trend in smoking prevalence among men aged 25-64 years-of-age during the period 1985-1992 in six Czech districts (n=25) participating in the MONICA project, whereas no significant changes in smoking prevalence were observed for women. Despite an average 0.7 annual decrease in standardized lung cancer mortality in men since the late 1980s, which has been predicted to continue through 2009, further increases in lung cancer mortality, estimated at 3.1% annually, are expected for the next decade among women (Kubik A, etal, Cas Lek Cesk, 10 May 1999;138(10):310-5).

It appears that women develop lung cancer after much less smoking exposure than men, and much earlier in life, regardless of their smoking history. This susceptibility of females to carcinogens may be explained by genetic factors. A gene, gastrin-releasing protein receptor (GRPr), located on chromosome X, that encodes a protein which is found on the surface of cells lining the lung, and is associated with a proliferative response of bronchial cells, and with long-term use of tobacco, is more active in women than in men. Women may be at increased risk because they have two copies of the GRPr gene, on each X chromosome, whereas men only have one copy, or because one GRPr gene in women is chronically active, even before exposure to nicotine, or both.

When stimulated by the GRP hormone, GRPr triggers cell proliferation typically seen in lung cancer. Researchers at the University of Pittsburgh Cancer Institute (Pittsburgh, PA), supported by a grant from the National Cancer Institute, observed an increase of the expression of GRPr in women, and also discovered that nicotine found in cigarettes stimulates expression of the GRPr gene in lung cells. By analyzing normal and malignant lung tissue, investigators found that 55% of nonsmoking women and 75% of women with ≤25 pack-years of smoking, expressed GRPr mRNA compared to none of the male nonsmokers, and only 20% of those with a ≤25 pack-year history. This research project did not measure exposure to passive smoke, that may also play a role in activating the GRPr gene in women which is being looked at in ongoing studies.

In contrast, p53 mutations are more prevalent among male lung cancer cases, but G to T tranversions appear to be more common in female cases. In a study conducted by the Finnish Institute of Occupational Health (Helsinki, Finland), INSERM U521 (Villejuif, France), and the International Agency for Research on Cancer (Lyon, France), 118 female and 161 male lung cancer cases were investigated using p53 mutations as a biomarker. Overall, p53 was mutated in 42% of the male and 29% of the female cases. Among males, when analyzed by tobacco smoke exposure, mutations were identified in 48% of ex-smokers and 46% of current smokers, but in none of nonsmokers. Among females, the results were 38%, 38%, and 18%, respectively. By histology, 52% of males and 37% of females with SCC carried a mutation while in adenocarcinoma, the corresponding values were 29% and 23%, respectively. G to T transversion, the mutation type associated with tobacco smoke exposure, represented 45% of the sequenced mutations among women, and 20% of those in males (Husgafvel-Pursiainen K, etal, AACR00, Abs. 143:22).

Recently, investigators at Harvard School of Public Health (Boston, MA) discovered that, after adjustment for environmental exposure, women with adenocarcinoma were more likely than men to harbor K-ras mutations, suggesting a possible role of estrogen exposure in either the initiation, or the selection of K-ras mutant clones in this histologic type. DNA from tumor tissue from 365 resected consecutive newly diagnosed patients with nsclc, was used to evaluate K-ras status using PCR-restriction fragment length polymorphism analysis. Codon 12 mutations in K-ras were detected in 22.1% of patients with adenocarcinoma, and in 4.8% of patients with other histologies. Among those with adenocarcinoma, codon 12 mutations in K-ras were found only in smokers, with women being 3.3 times more likely to have mutations than men, after adjustment for carcinogen exposure. Also, presence of the K-ras mutation was significantly associated with decreased survival in patients with Stage I disease, affording K-ras a prognostic value. The mutation remained a significant predictor of survival after the researchers adjusted for age, sex, and tumor stage. It is theorized that cells with K-ras mutations undergo clonal expansion *in vivo* through the action of secondary events, such as enhanced expression of growth factors, or alterations in genes such as those controlling cell cycle checkpoints. Estrogen may select for smoking-initiated clones, increasing the risk in women (Nelson HH, etal, JNCI, 1 Dec 1999;91(23):2032-8).

Age Factors in the Incidence of NSCLC

Although nsclc is a disease of the aged, increasingly it is diagnosed in younger patients. In a study conducted in Japan, medical records of 91 patients ≤40 years-of-age, and 3,221 >40 years-of-age with lung cancer were reviewed to compare smoking habits, distribution of histopathologic types, clinical stage, and survival. Results were as follows:

	Total #	Total (%)	Smokers (%)	Adenocarcinoma (%)	Advanced Disease (%)
>40 years-of-age	3,221	97.3			
Male			95	42	75
Female			39	73	
≤40 years-of-age	91	2.7			
Male			84	71	54
Female			39	92	
All Ages	3,312	100.0			

There was no difference in tumor size or survival between the two groups of female patients. Among males, advanced disease (Stages IIIb and IV) was more common in younger (75%) than in older patients (54%), but again, no survival difference was noted between the two groups (Sekine I, etal, Ann Thorac Surg, May 1999;67(5):1451-5). A similar finding was reported from a study of 126 (12.5%) patients <50 years old at diagnosis (median age=44 years), and 886 patients ≥50 years (median=65 years). Although younger patients presented with more advanced-stage disease, their overall survival was similar to that of older patients, suggesting that lung cancer is not inherently more aggressive in patients <50 years-of-age (Gadgeel SM, Chest, May 1999;115(5):1232-6). In other studies, however, advanced age was associated with higher death rates. In the EUROCARE study, 50% of patients <45 years old died within 1 year of diagnosis, increasing to almost 80% for those ≥75 years-of-age [Janssen-Heijnen ML, etal, Eur J Cancer, Dec 1998;34(14 Spec No):2191-6].

Stage at Diagnosis

Outcomes in nsclc are dependent on disease stage at diagnosis. Incidence rates by stage at first diagnosis vary based on the sophistication of the diagnostic work-up. Although there is little discrepancy in incidence of Stage IV nsclc at diagnosis, there is considerable variation in incidence estimates for Stage I and II and III disease. Often, patients are placed in the Stage I category, only to reclassified as Stage II after surgery when a closer look is possible.

In the USA, a large percent of nsclc is diagnosed in late stages, resulting in high mortality (see Exhibit 2). It should be noted here that mortality rates by stage vary from series to series. The data presented in Exhibit 2 is based on SEER data. Results from clinical trials reflect a higher rate of early-stage disease, but certain patient selection criteria probably favorably impact such data.

Survival

Survival depends on age, histology, stage at diagnosis and geographical location; the latter is a significant factor because locoregional healthcare programs may determine treatment options, and access to specialized care and, thus, influence survival. In a study to establish variations within Europe, regarding the prognosis of adult patients with lung cancer, by age, histology and country, survival analysis was carried out on 173,448 lung cancer cases, diagnosed between 1985 and 1989, from 44 population-based cancer registries participating in the EUROCARE study. Relative 1-year survival rates for patients with lung cancer varied from 24% to 40%, being highest in Finland, France, the Netherlands and Switzerland, and lowest in Denmark, England, Poland and Scotland. The 1-year survival for patients with nsclc remained more or less constant between 1978 and 1989 (25% in Denmark and 44% in Finland). Although some believe that the fairly large variation in lung cancer relative survival rates that exist between European countries is probably attributed to lack of specialized care (Janssen-Heijnen ML, etal, *ibid*) others claim that differences in treatment are unlikely to explain the findings. However, delays in diagnosing and treating patients in Denmark, compared with neighboring countries, could partially explain the lower patient survival experienced in that country (Storm HH, etal, Eur Respir J, Feb 1999; 13(2):430-5).

NSCLC Incidence by Histologic Type

Approximately 90% of all lung cancer is bronchogenic, i.e., it arises from stem cells; the remaining 10% of non-bronchogenic cancer may arise from alveolar cells (pneumocytes), glands and/or mesenchyme. Also, an overlap of expression profiles of small-cell lung cancer (sclc) and nsclc leads to the conclusion that both derive from the same stem cell (Petersen S and Petersen I, AACR00, Abs. 4321:680).

Although, currently, nsclc histology is not a determinant of treatment option, and survival outcomes do not appear to be different between histologic types, it is possible that this distinction will be important in treatment choice in the future, if drug therapies are tailored to treat these histologic types as unique entities. For instance, molecular biologic substaging of patients with Stage I nsclc demonstrated differential cancer-specific survival rates according to marker expression, gender, and histologic subtype (D'Amico TA, etal, Ann Thorac Surg, Mar 2000;69(3):882-6).

Among the four major classifications of nsclc (Exhibit 1), based on histologic attributes, SCC is still the most prevalent worldwide but has lost ground to adenocarcinoma in the USA. In the late 1980s, adenocarcinoma became the most common type of lung cancer among all sexes and races combined. Increases in the incidence of lung adenocarcinoma have been greater among women than men.

In Europe SCC remains the most common type. In a study conducted in Northern Finland that compared inci-

Exhibit 1
Histologic Classification of NSCLC and Estimated Incidence in the USA in 2000

Histology	Description	Total (%)	Total (#)
Squamous cell (epidermoid) carcinoma (SCC)	SCC is a centrally located endobronchial cancer commonly associated with lobar collapse, obstructive pneumonia, or hemoptysis; it is the classic form of lung cancer associated with cigarette smoking; SCC is also classified as hypertrophic, nodular and polypoid (Konaka C, etal, Br J Cancer, Jul 1999;80(9):1435-9)	31.2	41,025
Spindle cell variant	Also classified as pleomorphic or sarcomatoid carcinoma[1]		
Adenocarcinoma	Nonsquamous cell cancers are associated with early development of metastasis and are more likely to recur after surgical resection of Stage I tumors than other types of nsclc; adenocarcinoma is the lung cancer diagnosed in non-smokers; adenocarcinoma is classified as acinar, papillary, bronchoalveolar and solid tumor with mucin	50.0	65,640
Bronchoalveolar carcinoma (BAC)	BAC is a pathologically distinct type of nsclc that is more common in women and in nonsmokers than other histologic types of lung cancer; the incidence of BAC appears to be rising; BAC sometimes is associated with a distinct presentation and biologic behavior; it may present as a more diffuse lesion than other types of cancer; an infiltrate is present on chest radiographs of 30% to 40% of patients undergoing an attempt at surgical resection	10.0 to 25.0	6,564-16,420
Large-cell carcinoma	These tumors are usually detected when metastasized and are associated with poor prognosis; includes giant-cell carcinoma, also classified as pleomorphic or sarcomatoid carcinoma1 and clear-cell tumors of the lower respiratory tract	12.5	16,410
Other histologies[2]	These histologies include adenosquamous (mixed) carcinoma, and undifferentiated carcinoma	6.3	8,205
Total NSCLC		100.0	131,280
All Lung Cancer			164,100

[1] *In one study, although the sarcomatoid portions in all sarcomatoid carcinomas exhibited epithelial differentiation, there was no apparent difference in biologic behavior or prognosis between sarcomatoid and ordinary lung carcinoma; among 37 cases of sarcomatoid (spindle and/or giant cell) carcinoma of the lung, 5 cases (13.5%) comprised entirely of sarcomatoid components without carcinomatous elements while in the remaining 32 cases (86.5%) carcinomatous components were also present (Nakajima M, etal, Cancer, 15 Aug 1999;86(4):608-16).*

[2] *Another type of lung cancer is represented by mixed sclc and nsclc but its incidence is very low; among 2,806 cases of lung cancer diagnosed at the Ellis Fischel Cancer Center, University of Missouri and the Truman VA Hospital (Columbia, MO), between 1986 to 1998, 2,055 (73.2%) were nsclc, 727 (25.9%) sclc, and only 24 (0.8%) were mixed sclc and nsclc; survival in this latter type of lung cancer paralled that of nsclc rather than sclc (Raheem MA, etal, ASCO99, Abs. 1903:493a).*

dence by histologic type among 602 new lung cancer cases (male=85% and female=15%) who were diagnosed during the years 1990-1992, the annual incidence of lung cancer was 63/100,000 for males and 9.5/100,000 for females. SCC was the most common histologic type (40%), followed by adenocarcinoma (26%), sclc (24%), and large-cell carcinoma (4%). The incidence of lung cancer had decreased significantly among males, from 87/100,000 to 63/100,000, within 20 years, but increased among females from 4.1/100,000 to 9.5/100,000, chiefly because of a rise in the incidence of adenocarcinoma. Interestingly, these findings show an increase in the incidence of lung adenocarcinoma among females, a histologic type which is less closely related to smoking than the other cancers. Such findings may point to factors other than smoking as contributors to lung carcinogenesis (Makitaro R, etal, Eur Respir J, Feb 1999; 13(2):436-40).

Using the newly developed cDNA microarray hybridization technology, investigators identified gene expression patterns specific for adenocarcinoma and SCC. Genes commonly expressed in both tumor types included c-myc binding protein and transcription factor, glutathione S-transferase (GST), rho-GDP dissociation inhibitor, tissue inhibitor of metalloproteinase (TIMP)-1, vascular endothelial growth factor (VEGF), GRB2, fau, osteonectin/ basement membrane protein, and IL-1β precursor. Genes expressed in adenocarcinoma tissues only included vimentin, collagen 8/α1 and 16/α1, thrombospondin 2, and TIMP-3. Genes expressed in SCC tissues only included IGFBP2, urokinase-plasminogen activator (uPA), α1 and αE catenin, cytovillin 2, and integrin α3. Thus, dissimilarities between nsclc histologic types may provide an insight into the differences observed in clinical behavior (Young GD and Garver RI, AACR00, Abs. 4320:679-80).

When 64 lung SCC samples from 50 patients (25 each with or without evidence of metastasis) were investigated, chromosomal imbalances involved deletions most frequently on chromosomes 1p21-p31, 2q34-q36, 3p, 4p, 4q,

5q, 6q14-q24, 8p, 9p, 10q, 11p12-p14, 13q13-qter, 18q12-qter and 21q21. DNA over-representations were most pronounced for chromosomes 1q11-q25, 1q32-q41, 3q, 5p, 8q22-qter, 11q13, 12p, 17q21-q22, 17q24-q25, 19, 20q and 22q. Deletions at 3p12-p14, 3p21, 4p15-p16, 6q24-qter, 8p22-p23, 10q21-qter and 21q22, as well as over-representations at 1q21-q25, 8q, 9q34, 14q12 and 15q12-q15, occurred significantly more often in the metastatic tumor group. A comparison of paired samples confirmed these findings in individual cases, suggesting distinct genetic changes, in particular extension of small interstitial deletions during tumor progression. Importantly, metastasis-associated lesions were frequently detectable in the primary tumor providing a method of identifying patients at risk for tumor dissemination (Petersen S, etal, Br J Cancer, Jan 2000;82(1):65-73).

Incidence of adenocarcinoma has risen steadily in the USA, particularly, that of bronchioloalveolar carcinoma (BAC), a pathologically distinct type of adenocarcinoma of the lung that is more common in women, and in nonsmokers, than other histologic types of lung cancer. Patients with BAC have clinical, radiographic, and pathologic characteristics that distinguish them from patients with other types of nsclc.

BAC has a favorable prognosis when adjusted for age and extent of disease. Recurrence and survival rates of early-stage BAC (T1-N0) are similar to those of SCC, and better than those of non-BAC adenocarcinoma. However, T1-N1/T2-N0 and Stage II and III BAC recurs more frequently than either SCC, or non-BAC adenocarcinoma, and Stage II and III BAC is associated with a higher mortality rate than does SCC or non-BAC adenocarcinoma (Grover FL and Piantadosi S, Ann Surg, Jun 1989; 209(6):779-90). In a study conducted at the National Naval Medical Center (Bethesda, MD), investigators collected clinical, radiographic, and pathologic information on 28 patients (women=12) with Stage IIIb and IV BAC, and compared this data with that of 124 patients (women=40) with other histologic types of nsclc. Nine (32%) of the patients with BAC had never smoked cigarettes, compared to 20 (16%) controls. Regarding presentation, 18 (64%) patients with BAC had bilateral multilobar, or multicentric pulmonary involvement, compared with 13 (15%) controls. Patients with advanced BAC (Stage IIIb and IV) survived a median of 15 months from the time of diagnosis, compared to 10 months for controls. Patients with advanced-stage BAC are more likely to present with bilateral diffuse pulmonary involvement, are less likely to develop brain metastases, and live longer than patients with other types of Stage IIIb and IV nsclc (Breathnach OS, etal, Cancer, 1 Oct 1999;86(7):1165-1173).

TUMOR MARKERS

Numerous markers have been associated with lung cancer (see Exhibit 3). Markers may be used in risk assessment, screening, diagnosis, disease staging, prognosis, treatment and disease monitoring. However, although the list of markers grows daily in size, identification of markers that can be used in the clinic remains elusive. Also, it appears that certain markers may be possess a racial, ethnic or gender preference, further complicating an already complex situation. However, once established, their value would be immense in preventing cancer in individuals predisposed to the disease, in personalizing treatment for those afflicted, and in the early detection of disease progression.

Hereditary Factors

A hereditary link was found between relatives with lung cancer among smokers and, recently, among nonsmokers with lung cancer. Genetic susceptibility to various cancers is evident in families with cases of lung cancer in nonsmokers which may assign a hereditary factor in this disease. For instance, having a first-degree relative with cancer approximately doubles the risk of lung cancer among nonsmokers. Also, in a cohort analysis of relatives, excess risk was evident in relatives of lung cancer cases, indicating that nonsmokers with lung cancer may be genetically predisposed to cancer. A population-based case-control study (Mayne ST, etal, Cancer Epidemiol Biomarkers Prev, Dec 1999;8(12):1065-9), conducted by investigators at Yale University School of Medicine (New Haven, CT) and the Foundation for Blood Research (Portland, ME), in 437 (female=218) nonsmoking men and women (never smokers=45%, and former smokers who had quit at least 10 years before diagnosis/ interview=55%) with lung cancer, and 437 matched controls, in New York State, between 1982 and 1984, found that those with lung cancer were significantly more likely than controls to report having:

- a paternal history of any cancer [odds ratio (OR)=1.67], and aerodigestive tract cancers (OR=2.78)
- a maternal history of breast cancer (OR=2.00)
- a history of any cancer in brothers (OR=1.58), and sisters (OR=1.66)
- a nearly significant excess of lung cancer (OR=4.14), aerodigestive tract cancer (OR=3.50), and breast cancer (OR=2.07) in sisters

The highly polymorphic Hras1 variable number of tandem repeats (VNTR), which maps 1 kb downstream from the human Hras1 gene, has been described as an inherited predisposing factor in many human cancers. Rare Hras1 VNTR alleles are associated with increased lung cancer risk; when the Hras1 VNTR was genotyped in 295 lung cancer patients, and 500 healthy controls, 35 Hras1 VNTR alleles were found, of which 24 were defined as rare. The gender-independent risk rate, associated with rare alleles, was 3.3; when a matched control group was used, the risk rate was 12.7 for individuals with rare alleles at the Hras1 VNTR locus. There was a low frequency (4.7%) of microsatellite alterations in lung tumors, but the frequency of altered microsatellite loci was higher in patients with rare Hras1 VNTR alleles than in those with common alleles.

Exhibit 2
Staging of NSCLC

Stage	TNM Classification	Description
Occult disease	Tx, N0, M0	Nonassessable tumor; presence of malignant cells in sputum and bronchial washings but snot visible with bronchoscopy or imaging
Stage 0	Carcinoma *in situ*	Noninvasive tumors incapable of metastasizing
Stage I	10%-25% of patients are placed in Stage I; 5-year survival is 50%	
Stage Ia	T1, N0, M0	Tumor ≥3 cm in greatest dimension with no bronchoscopic evidence of invasion more proximal than the lobar bronchus, or uncommon superficial tumor of any size with its invasive component limited to the bronchial wall, which may extend proximal to the main bronchus (T1)
Stage Ib	T2, N0, M0	Tumor >3 cm, and/or one that involves the main bronchus, and/or has invaded the visceral pleural, and/or associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the whole lung (T2)
Stage II	10%-20% of patients are placed in Stage II; 5-year survival is 29%	
Stage IIa	T1, N1, M0	T1 and metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes (N1)
Stage IIb	T2, N1, M0; T3, N0, M0	Tumor of any size that has invaded the chest wall, diaphragm, mediastinal pleura, parietal pericardium, or tumor in the main bronchus <2 cm distal but not involving the carina, s or atelectasis pneumonitis of the whole lung (T3)
Stage III	20%-25% of patients are placed in Stage III; 5-year survival is 10%	
Stage IIIa	T1, N2, M0; T2, N2, M0; T3, N1, M0; T3, N2, M0	T1 or T2 and N1 or metastasis in ipsilateral mediastinal and/or subcarinal lymph nodes (N2); or tumor of any size that has invaded the chest wall, diaphragm, mediastinal pleura, parietal pericardium, or tumor in the main bronchus <2 cm distal but not involving the carina, or atelectasis pneumonitis of the whole lung (T3) and N1 or N2
Superior sulcus tumors	T3, N0 or N1, M0	Locally invasive disease usually with a reduced tendency for distant metastases
Chest wall tumors	T3, N0 or N1, M0	Bulky primary tumors that directly invade the chest wall
Stage IIIb	Any T, N3, M0; T4, any sN, M0	T1, or T2, or T3, and metastases in contralateral mediastinal, or hilar, and ipsilateral or contralateral scalene, or supraclavicular lymph nodes (N3); or any N and tumor of any size that invades the mediastinum, or heart, great vessels, trachea, esophagus, vertebral body, carina; or separate tumor nodules in the same lobe; or tumor with a malignant pleural effusion attributed to tumor*
Stage IV	Any T, any N, M1	Distant metastasis or intrapulmonary ipsilateral metastasis in a lobe other than the lobe containing the primary lesions (M1); 40% of patients are placed in Stage IV; 5-year survival is 20%

Source: Revised staging adopted by the American Joint Committee on Cancer (AJCC) and the Union Internationale Centre le Cancer (UICC)

** Most pleural effusions associated with lung cancer are attributed to tumor. However, there are a few patients in whom multiple cytopathologic examinations of pleural fluid are negative for tumor. In these cases, fluid is non-bloody and is not an exudate. When these elements and clinical judgment dictate that the effusion is not related to the tumor, it should be excluded as a staging element, and the patient should be staged as T1, T2, or T3.*

These findings point to a genetic mechanism which increases allelic diversity (Lindstedt BA, etal, Anticancer Res, Nov-Dec1999;19(6C):5523-7). Previously, in tumor samples from 214 lung cancer patients and 309 unaffected controls, 22 different alleles of Hras1 were detected, which were grouped according to their frequencies as common, intermediate, and rare; frequency of rare alleles in lung cancer patients (16/428) was significantly different from that in the control group (5/618), and cases with rare alleles were at a 4.7-fold greater risk of lung cancer than those with no rare alleles (Ryberg D, etal, Environ Health Perspect, Nov 1992;98:187-9).

Using peripheral blood samples and PCR-based detection methods, a higher percentage (32.7%) of rare HRAS1 VNTR alleles were confirmed in lung cancer patients than in unaffected controls (21.9%), among 466 HRAS1 VNTR alleles from 233 lung cancer patients, and 892 alleles from 446 unaffected controls. Presence of rare alleles was associated with an increased risk (OR=1.68), of lung cancer indicating a genetic predisposition. No differences based on other clinicopathologic variables were observed. A meta-analysis showed a higher distribution of rare alleles among Caucasian Spaniards than in American and Northern European Caucasian populations. Interracial variations in allele frequencies and variations between Caucasian subpopulations suggest that genetic variations may be involved in susceptibility to lung oncogenesis, especially in certain ethnic groups (Rosell R, etal, Clin Cancer Res, Jul 1999;5(7): 1849-54).

Minisatellite sequence within the Hras1 proto-oncogene locus was allelotyped at the Russian Academy of Medical Sciences in Moscow, in 60 patients with lung adenocarcinoma, analyzed with respect to the effect of tobacco, and results were compared with analogous data from patients with SCC, and healthy individuals. In contrast to the patients with SCC, the frequency of the Hras1 locus rare alleles in patients with lung adenocarcinoma was higher than in individuals without cancer; it was significantly higher (17.6%) for nonsmokers with cancer than in healthy individuals(2.7%) Higher frequencies of the common a4 allele were observed in smokers with adenocarcinoma. These findings point to the combined effect of endogenous and exogenous factors on the development of lung adenocarcinomas in humans (Gaspar'ian AV, etal, Genetika, Nov 1998;34(11):1537-41).

Other Biomarkers

Numerous biomarkers have been linked to nsclc (Exhibit 3). However, few have yet to be proven useful in the clinic as diagnostic or prognostic factors or as targets for drug development. Nevertheless, the role of these markers is being elucidated in complex pathways that may prove critical in understanding tumorigenesis and metastasis, and in arriving at treatment approaches that take into account all contributors to these events on an individualized basis.

After several libraries of differentially expressed genes in lung cancer were cloned at the Institute of Pathology (Berlin, Germany) using suppression subtractive hybridization (SSH), expression of these genes in lung carcinoma cell lines were compared to that in normal airway epithelial cells. The more than 1,300 sequenced clones encoded cell adhesion and communication proteins, cytoskeletal proteins, transcription factors, and well characterized oncogenes and antioncogenes. Using a panel of sclc and nsclc cell lines, differential expression of more than 60% of the cloned cDNA fragments was confirmed. Out of a majority of expressed sequence tags (EST) of yet unknown function, the full length cDNA and gene structure of 3 new genes were characterized (Petersen S and Petersen I, AACR00, *ibid*).

DIAGNOSIS AND SCREENING

Diagnostic approaches in lung cancer were described in FO, pp 1080-1084. Conventional chest x-ray is the most commonly used technique for diagnosing lung cancer. A variety of *in vitro* and *in vivo* approaches are being evaluated in the specific diagnosis of nsclc.

Noninvasive Imaging

Noninvasive imaging is used in the screening and diagnosis of lung cancer, in general (see FO, p 1080-84), and to detect distant metastases in nsclc, in particular. Noninvasive imaging techniques used in the detection of metastases include brain and abdomen CT scans, brain MR scans, scintigraphy (see FO, pp 1112-14), and radionucleotide bone scans.

Electron beam tomography (EBT), an ultrafast CT approach developed by Imatron (South San Francisco, CA), was awarded 510k market clearance in April 2000 for the diagnosis of lung abnormalities. The rapid data acquisition capability of the EBT scanner allows physicians to perform a low-dose, 15 second, 140 slice, single-breath hold scan of the complete lung volume. The inherent ultrafast scan speed of the Imatron EBT scanner also reduces motion artifacts within the lung, which originate from the complex motion of the beating heart. EBT scanning of the lung can be used to diagnose a wide variety of lung anomalies, including small lung nodules. Imatron's EBT scanners are currently in use at more than 120 major medical facilities and imaging centers around the world.

Positron emission tomography (PET) in lung cancer diagnosis was discussed in FO, pp 1083-1084 and 1114.

In Vitro Approaches

In vitro approaches, represent the most desirable means of screening, diagnosis, staging and prognosis. Genetic alterations have been detected in the plasma or serum DNA that correlate with histologically proven advanced cancer. The presence of these markers, however, may also be related to early disease, providing a simple screening method. Although no such tests are currently available in the USA in nsclc, several described below show promise.

Cell-associated transferrin receptor (TfR) may help in the differential diagnosis of lung cancer versus pneumonia. A soluble form of TfR (sTfR) in human serum was shown to be proportional to the number of cellular TfRs. When sTfR in the serum and bronchoalveolar lavage (BAL) fluid of patients with lung cancer (n=32), and patients with chronic obstructive pulmonary disease (n=22) were measured, there was no difference in serum sTfR between the cancer and COPD groups, but a higher level of cell-associated TfR was found in BAL of nsclc than COPD. There was no correlation between BAL cell-associated TfR and tumor size, nodal status, presence of metastases, and serum sTfR. BAL cell-associated TfR was negatively correlated with BAL supernatant neuron-specific enolase (NSE). A combination of BAL supernatant NSE and cell-associated TfR detected lung cancer with a sensitivity of 91%, a specificity of 59%, and positive and negative predictive values of 81% and 71%, respectively (Dowlati A, etal, Br J Cancer 1997;75(12):1802-6).

Cyfra 21-1, that detects soluble cytokeratin 19 fragments in serum, is one of the leading markers in nsclc because of its high specificity and sensitivity (63%). Investigators at Klinikum Grosshadern at Ludwig-Maximilians University (Munich, Germany) investigated the diagnostic value of Cyfra 21-1 in the detection of recurrent disease in 86 nsclc patients following radical resection (R0), followed-up for a median period of 22.7 months. Preoperatively, Cyfra 21-1 was positive (cut off was 3.3

ng/ml) in 38/86 (45%) patients; 48 hours after surgery Cyfra 21-1 concentrations in all 38 patients were within the reference range corresponding to an R0 resection. During further follow-up, all 22 patients who developed local recurrence and/or distant metastases had elevated Cyfra 21-1 values at the time of detection of relapse and, in 8 of these patients, Cyfra 21-1 increase preceded detection of recurrence by 2 to 15 months. The 16 patients who remained disease-free had stable low Cyfra 21-1 values all the time. Among 48 patients who were Cyfra 21-1-negative preoperatively, 15 developed recurrent disease; 7/15 expressed cytokeratin 19-fragments. Therefore, Cyfra 21-1 possesses a high specificity and sensitivity in the detection of recurrent nsclc in patients with elevated Cyfra 21-1 values at time of primary diagnosis, and may prove an economical contributor to follow-up care (Stieber P, etal, Anticancer Res, Jul-Aug 1999;19(4A):2665-8).

Cyfra 21-1 is available as a radioimmunoassay (RIA), and sandwich enzyme-linked immunosorbent assay (EIA). Cyfra 21-1 is sold in certain European countries and Japan by various suppliers including Fujirebio (Tokyo, Japan) and Roche Diagnostics (Mannheim, Germany), among others.

Cyfra 21-1 may also be applicable in disease monitoring. In a prospective study of 48 consecutive patients with Stage IIIB/IV nsclc, Cyfra 21-1 was found to be elevated in 29/48 (60.4%) patients prior to therapy, and in 10/48 (20.8%) patients at tumor progression. The most striking result was detection of progressive disease by rising marker levels. Except one case, there was no false-positive elevation of Cyfra 21-1 levels (Ebert W and Muley T, Anticancer Res, Jul-Aug 1999;19(4A):2669-72).

DR-70, an ELISA developed by AMDL (Tustin, CA) that uses affinity purified rabbitt anti-DR70 polyclonal antibody, was approved and launched in Canada. Originally referred to as ring-shaped particle tumor marker, the exact nature of DR-70 has not been elucidated. Investigators at Hubei Medical University (Wuchang, Wuhan, China) conducted a clinical study using DR-70 immunoassay for the detection of 13 different cancers in 277 healthy subjects, and 136 cancer patients. Results showed that the DR-70 immunoassay kit was capable of detecting cancers with high degree of specificity and sensitivity. At 95% specificity level, the sensitivity of the assay was 87.8%, 92.6%, 65.2% and 66.7%, respectively for lung, stomach, breast and rectum cancers. Furthermore, test kits were shown to be stable and performed reproducibly (Wu D, etal, J Immunoassay, Feb 1998;19(1):63-72). A similar study was conducted at the Cross Cancer Institute (Edmonton, Alberta), between in 1993 and 1995, involving 237 sclc and nsclc patients, and 244 normal controls. Overall sensitivity of the assay was 66% at a specificity of 92%.

Gastrin-releasing protein receptor (GRPr) gene expression may also lead to the development of an *in vitro* test to assess lung cancer risk because GRPr expression in circulating blood cells mirrors that seen in lung cells.

Heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1, an RNA binding protein that is required for the maturation of mRNA precursors, was one of the first markers whose overexpression appeared to render it predictive of lung cancer. Although hnRNP A2/B1 is required in small amounts by most normal active cells, its overexpression in exfoliated epithelial cells that are coughed or washed from the lungs that have lost adhesion, is remarkably predictive of lung cancer. This, however, is not the case in cells obtained from biopsies or resected epithelium. Based on immunologic analyses, performed both in lung and non-lung cancer cell lines, expression of hnRNP A2/B1 was not specific for lung cancer (Satoh H, etal, Int J Oncol, Apr 2000;16(4):763-7).

The difference in expression of hnRNP A2/B1 in cells obtained from lung tumor biopsies from that detected in exfoliated tumor cells was illustrated in a clinical trial that used entrants from a randomized retinoid chemoprevention trial. When 1,078 available biopsy specimens, 147 individuals at baseline, and 68 individuals who completed the intervention, were analyzed, overexpression of hnRNP A2/B1 was frequently detected in normal (41%) and abnormal (37%) bronchial epithelium. There was no correlation between hnRNP A2/B1 overexpression, and different histologic changes. In cases with hnRNP A2/B1 overexpression, immunoreactivity was homogeneously expressed in all biopsied sites. For the 68 cases with serial biopsies, there was no significant modulation of hnRNP expression by retinoid intervention or smoking status (Zhou J, Clin Cancer Res, Jul 1998;4(7):1631-40).

In initial investigations, the accuracy of this biomarker, detected using MAb 703D4 in 62 archived dysplastic (but not diagnostic) specimens collected 2 years in advance of clinical lung cancer, was 88% (Zhou J, etal, J Biol Chem 1996;271:10760-66). Subsequently, the predictive value of hnRNP A2/B1 overexpression was prospectively assessed in two high-risk populations, 595 patients with Stage I, resected lung cancer, for whom the annual risk of second primary lung cancer is 1-5%, and 6,285 Chinese tin miners with extensive exposure to tobacco smoke, radon, and arsenic, among whom the annual incidence of lung cancer is 1%. In these two populations, hnRNP A2/B1 overexpression predicted lung cancer in 67% and 69% of cases, respectively, a 35-fold and 76-fold improvement in positive predictive value over background cancer risks of 2.2% and 0.9%, respectively (Tockman MS, etal, Clin Cancer Res 1997;3:2237-46).

The predictive value of overexpression/up-regulation of hnRNP A2/B1 was assessed in a prospective study conducted at Roy Castle International Centre for Lung Cancer Research (Liverpool, UK), involving 103 individuals suspected of having lung cancer, and was compared with routine diagnostic cytology. Using MAb 703D4, hnRNP expression was analyzed, in a blinded study, in individuals with metaplastic bronchial epithelial cells, or tumor cells in bronchial lavage specimens. A full clinical work-up was also undertaken, including bronchoscopy, and radiologic

Exhibit 3
Biomarkers Associated with NSCLC

Adenomatous polyposis coli (APC) gene
Allelic deletions of APC gene are frequent in advanced stages of lung cancer, and have been correlated with poor prognosis; when 33 cases of nsclc (22 SCC and 11 adenocarcinomas) were investigated for LOH at 5q21, LOH was detected in 4/9 (44%) informative adenocarcinomas, and in 13/16 (81%) SCC; LOH was frequent in early stages (12/15 Stage I cases), and did not correlate with recurrence, or poor survival; LOH was more frequent in SCC than in adenocarcinoma, was frequent in early stages of the disease, but did not have prognostic significance (Sanz-Ortega J, etal, Pathol Res Pract 1999;195(10):677-80). A correlation between methylation status of the APC gene promoter region, and APC gene expression, has been noted in nsclc, among 19 nsclc samples (adenocarcinomas=13, SCC=6), obtained from surgically resected specimens, and matched normal controls, a significantly higher methylation status of the APC promoter region was observed in tumor tissue compared to controls; hypermethylation did not occur in all low APC expressors, but all patients with very high APC expression levels showed no promoter methylation; survival was superior in patients with a high level of APC gene expression with a 3-year survival of 72.7% in the high-expression group compared to 37.5% in the low-expression group; MST was 59.7 months and 24.7 months, respectively; it appears that APC promoter methylation is involved in the progression of nsclc, and could be a regulatory mechanism of APC gene expression; in addition, APC gene expression could be a predictive marker for long-time survival (Jan Brabender J, etal, AACR00, Abs. 3176:498)
Bax
Among 203 nsclc tissue samples, 146 (71.9%) were bax+ (Huang C, etal, Am J Pathol, Sep 1999;155(3):955-65 and ASCO99, Abs. 1902:493a). Overexpression of bax in cultured cell lines from human lung carcinoma activates caspases, induces apoptosis, and suppresses cell growth (Kagawa S, etal, Cancer Res, 1 Mar 2000;60(5):1157-61). High expression of bax was detected in formalin fixed and paraffin embedded lung cancer tissues in 72.7% of a cohort of 55 patients with nsclc; there was no correlation between bax expression and any clinicopathologic parameters (sex, age, TNM status, tumor grade, or histologic type); overexpression of bax reflects the "apoptotic tendency" of cells during neoplastic proliferation but the role of bax in nsclc pathogenesis is unclear (Caputi M, etal, Anticancer Res, Jan-Feb 1999;19(1B):825-7)
Bcl-2
Among 203 nsclc tissue samples, 79 (38.9%) were bcl-2+ (Huang C, etal, *ibid*). Expression of bcl-2 was detectable in 39/84 (46%) nsclc with the percentage of bcl-2-positive cases varying according to histologic type; positive bcl-2 immunostaining was observed in 27/46 SCC (59%), 7/25 adenocarcinomas (28%) and 5/13 large cell carcinomas (38%); the frequency of positive bcl-2 expression in SCC was significantly higher than that in the other two types; there were no significant differences in the frequency of bcl-2 expression and gender, T and N factors, or TNM stage; patients with bcl-2-negative tumors experienced significantly shorter survival times than those with bcl-2-positive tumors; in univariate analysis of various potential prognostic factors only TNM stage and bcl-2 status were significant prognostic factors; in multivariate analysis bcl-2-negative status was an independent unfavorable prognostic factor (Laudanski J, etal, Neoplasma 1999;46(1):25-30)
c-K-ras located on chromosome 12p12.1
A high frequency of K-ras mutations may point to preneoplastic changes in the bronchial epithelium as a result of genotoxic injury; up to 30% of adenocarcinomas exhibit K-ras mutations (Slebos RJ and Rodenhuis S, JNCI Monogr 1992;13:23-29). Mutations and overexpression of K-ras correlate with decreased survival, particularly in resectable disease (Salgia R and Skarin AT, J Clin Oncol 1998;16:1207-1217). When K-ras codon 12 mutations in a group of 52 bronchoalveolar lavage specimens from patients at risk of a second lung cancer were analyzed, 84% contained at least one mutation (Scott FM, etal, Clin Cancer Res, Mar 1997;3(3):479-82)
Cadherins (E-cadherin and P-cadherin)
Expression of cadherins and catenins is often altered in nsclc when compared to the progenitor bronchial epithelium; these alterations may play a role in the development of a malignant phenotype (Smythe WR, etal, Lung Cancer, Jun 1999;24(3):157-68)
Cyclooxygenase 2 (COX-2)
In a cohort of 130 resected adenocarcinoma patients, immunohistology confirmed presence of tumor cells with significantly increased COX-2 immunoreactivity in 93/130 (72%) cases; however, no relationship was found between increases in COX-2 expression and clinical outcomes in the entire cohort, but a significant relationship was observed between elevated COX-2 expression and shortened patient survival in a cohort of patients with Stage I adenocarcinoma (Achiwa H, etal, Clin Cancer Res, May 1999;5(5):1001-5)
Cell-cell adhesion molecule (C-CAM) and isoforms L-form C-CAM1 and S-form C-CAM1
When paired tumors and corresponding normal lung tissues from 51 patients with nsclc and 43 cell lines were analyzed to determine expression profiles of L-form C-CAM1 and S-form C-CAM1, investigators at the M. D. Anderson Cancer Center found that L-form C-CAM1 was predominant (38/51 or 75%) in normal lung tissues, while most (43/51 or 84%) of primary nsclc tissues expressed predominantly S-form C-CAM1; similarly, 19/24 (79%) nsclc and 17/20 (85%) sclc cell lines expressed predominantly S-form C-CAM1; frequent alteration of the C-CAM1 expression pattern suggests an important role in lung tumorigenesis (Wang L, etal, AACR00, Abs. 4954:779-80)

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Cyclin D1 (CD1) on chromosome 11q13

CD1 expression appears to be a favorable prognostic factor based on a univariate analysis of samples from 77 nsclc patients resected between 1990 and 1995; CD1 was expressed in 13 (11.7%) of 111 nsclc, but the CD1 gene was neither significantly amplified nor rearranged; CD1 expression significantly correlated with altered p53 protein expression, but not with p16 and Rb protein status; although proliferative activity was higher in CD1+ tumors than in CD1- tumors (this difference was not statistically significant), patients with CD1+ tumors survived longer than those with CD1- tumors, with 5-year survival rates of 89% and 64%, respectively (Mishina T, Br J Cancer, Jun 1999;80(8):1289-95). Amplification of the CD1 locus was detected in 14/298 (5%) nsclc specimens; all 12/12 specimens with amplification of the CD1 gene for which RNA was available, were found to express the CD1 transcript, and 11/12 overexpressed the transcript to levels higher than that of normal lung tissue; CD1 gene amplification was associated with advanced lymph node involvement, but not with larger tumor size, or adverse outcome; CD1 gene amplification and overexpression occurred independently of rb gene inactivation, but tumors with amplification of the CD1 gene were more likely to have EGFr gene amplification (Reissmann PT, etal, J Cancer Res Clin Oncol 1999;125(2):61-70)

DICE1 located on chromosome 13q14.12-14.2

When compared to normal lung tissue, expression of DICE1 mRNA was either reduced or undetectable in the majority of nsclc; location of the DICE1 gene in the region of allelic loss, its high evolutionary conservation, and the downregulation of expression in carcinoma cells, suggests that DICE1 is a candidate tumor suppressor gene in nsclc (Wieland I, etal, Oncogene, 12 Aug 1999;18(32):4530-7)

E-selectin

High serum E-selectin levels in nsclc, especially with carbohydrate antigen-positive disease, may be associated with a poor prognosis; serum E-selectin levels of 101 patients with resected nsclc were measured on admission to be 48.9±25.7 ng/ml; the E-selectin-positive rate was 22.7%, being correlated with the progression of T-factor; generally, survival rate was significantly lower among those with high levels of serum E-selectin compared to those with normal E-selectin levels; this finding was confirmed by multivariate analysis; in 52 cases, the mean postoperative E-selectin level (36.93 ng/ml) was significantly lower than the preoperative E-selectin level (43.57 ng/ml), indicating that certain nsclc may induce expression of E-selectin; survival curves were negatively affected by expression of two carbohydrate antigens, Sialyl Lewisx (SLX) and Sialyl Lewisa (CA19-9), which were detected in 65/101 resected nsclc specimens; survival curve was significantly worse in cases within the high E-selectin group expressing these antigens than in the normal E-selectin group; however, there was no significant difference in the survival curve between the high and normal E-selectin groups when carbohydrate antigens were negative (Tsumatori G, etal, Jpn J Cancer Res, Mar 1999;90(3):301-7)

EGFL6

EGFL6, a gene discovered by Hyseq (Sunnyvale, CA) scientists, is expressed in brain and lung tumors but not in normal adult tissue; it appears that EGFL6 gene encodes an extracellular signal peptide (Yeung G, etal, Genomics, 1 Dec 1999;62(2):304-7)

E1AF/PEA3

E1AF/PEA3 is a member of the ETS-related transcriptional factor family; when E1AF expression was analyzed in 19 cases of resected nsclc, 12/19 tumors showed E1AF expression; furthermore, E1AF expression was present in cancer cells but not in concomitant interstitial cells (Hiroumi H, AACR99, Abs. 878:132)

ErbB-2, erbB-3 and erbB-4 receptors

When differential expression of p185erbB-2, p160erbB-3 and p180erbB-4, and their ligand heregulin α, was examined in normal bronchial epithelial, and nsclc cell lines, expression of p185erbB-2 and p160erbB-3 varied from very low to a high level in nsclc cell lines, and a low level in normal bronchial cells; in contrast, p180erbB-4 was detected only in nsclc cell lines, but not in normal bronchial cells, while heregulin α was expressed at intermediate levels in both normal and cancer cell lines; erbB-2, erbB-3 or erbB-4 receptors were constitutively tyrosine phosphorylated in lung cancer cell lines, but only erbB-2 and erbB-3 were autophosphorylated in normal cells; therefore, constitutive activation of erbB-2, erbB-3 and erbB-4 receptors could be induced by heregulin α via an autocrine loop mechanism, and the active forms of erbB-4 may cooperate with the other members of the EGFr family in human lung carcinogenesis (al Moustafa AE, etal, Anticancer Res, Jan-Feb 1999;19(1A):481-6). ErbB-2/HER2 is overexpressed in a significant fraction of lung carcinomas in a manner that correlates with poor prognosis; oncogenicity of ErbB-2 in human epithelia may not rely on the existence of a specific ligand but, rather, on its ability to act as a coreceptor for multiple stroma-derived growth factors (Klapper LN, etal, PNAS USA, 27 Apr 1999;96(9):4995-5000)

Fas (CD95)/APO-1 and Fas ligand (FasL)

Although nsclc cells frequently express Fas, the Fas pathway is impaired, apparently by labile protein inhibitors; also, only a small fraction of FasL+ cell lines exhibit functional FasL molecules (Nguyen DM, etal, ACR00, Abs. 4408:693). Based on analysis of proteins of Fas and FasL in 164 nsclc tumor samples, patients with Fas+ tumors survived significantly longer than those with Fas- tumors; in contrast, FasL did not significantly influence patient survival; based on a multivariate analysis, lymph node status, and Fas expression were significant prognostic factors; incidence of lymph-node involvement was significantly higher in both Fas- and FasL- patients compared to those positive; also, Fas+ and FasL+ carcinomas demonstrated a greater sensitivity to doxorubicin *in vitro* (Koomagi R and Volm M, Int J Cancer, 21 Jun 1999;84(3):239-43)

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GRP receptor (GRPr)

Activation of GRPr in the airway has been associated with a proliferative response of bronchial cells to GRP, and with long-term use of tobacco; GRPr, because of its location on the X chromosome, is more frequently expressed in female nonsmokers, and activated earlier in women than in men exposed to tobacco; expression of a pair of GRPr genes in women may explain their higher susceptibility to tobacco-induced lung cancer (Shriver SP, etal, JNCI, 5 Jan 2000; 92(1):24-33)

Insulin-like growth factors (IGF)-I and II, and IGF-binding protein (IGFBP)-3

IGFs, in particular IGF-I and IGF-II, strongly stimulate the proliferation of a variety of cancer cells, including lung cancer cells; plasma levels of IGF-I are higher, and those of IGFBP-3 lower in patients with lung cancer as compared to controls; in a case-control study, investigators at M. D. Anderson Cancer Center examined the possible involvement of IGFs in the development of lung cancer, by comparing IGF-I, and II, and IGFBP-3 levels in the plasma of 178 newly diagnosed lung cancer patients, and 199 matched controls; mean IGF-I levels were significantly higher in the cancer cases (165 ng/ml) than in controls (143 ng/ml), with the difference being most evident in Hispanics (154 ng/ml versus 104 ng/ml) and African Americans (214 ng/ml versus 144 ng/ml); IGF-I levels were also higher in females than in males, and were slightly higher in smokers than nonsmokers; IGF-II and IGFBP-3 levels did not differ between cancer cases and controls (Yu H,etal, AACR98, Abs. 4281:629). In a similar case-control study, plasma levels of IGF-I, IGF-II, and IGFBP-3 were measured in 204 consecutive patients with histologically confirmed primary lung cancer, and 218 matched (by age, sex, race, and smoking status) controls; high plasma levels of IGF-I were associated with an increased risk of lung cancer, but there was no such association with plasma IGFBP-3 levels, unless adjusted for IGF-I level; however, taken together, high plasma levels of IGFBP-3 were associated with reduced risk of lung cancer; IGF-II was not associated with lung cancer risk (Yu H, etal, JNCI, 20 Jan 1999;91(2):151-6)

IGF-1 receptor (IGF-1r)

IGF-1r levels in two sublines (H-59 and M-27) of the Lewis lung carcinoma, expressing high and low levels of IGF-1r, respectively, correlated with lung cancer metastasis to the liver; also, when M-27 carcinoma cells were transfected with a plasmid vector that expressed a full-length human IGF-1r cDNA, and expression of the human receptor in the stable transfectants was confirmed, cells exhibited an enhanced proliferative response to IGF-1, an increased clonogenic potential, and acquired an invasive potential; when inoculated via the splenic/portal route *in vivo*, these cells, but not mock-transfected cells, gave rise to multiple tumor nodules (Long L, etal, Exp Cell Res, 10 Jan 1998;238(1):116-21)

MLH1 and MSH2

Inactivation of genes hMLH1 and hMSH2 is a common event in the development of nsclc; 88/150 (58.6%) nsclc specimens exhibited reduced expression levels of hMLH1 protein and 85/147 (57.8%) of hMSH2 protein; reduced expression levels of both proteins were observed in 51/150 (34%) specimens; in adenocarcinomas, reduction of hMSH2 expression was more frequently observed than hMLH1, while the reverse was observed in SCC; reduced expression of hMLH1 was more frequently associated with heavy smokers, both in terms of daily tobacco uptake and total smoking exposure (pack-years); an association between hMLH1 reduced expression and nodal metastasis in SCC was also observed; there were no mutations in the examined promoters, or exons, in these two genes; an allelic loss appears to be a major genetic event in hMLH1 silencing while a putative negative regulator of hMSH2 gene may be located at locus 3p14 (Liloglou T, etal, AACR00, Abs. 1205:188-89)

MUC1 (episialin)

When 93 tumor samples from patients with early stage nsclc, treated with surgery alone, were examined for episialin, EGFr, and c-erbB-2, episialin depolarized expression did not correlate with any histopathologic variables (stage, grade, histology, and Ki67 proliferation index); no correlation was observed between episialin and EGFr or c-erbB-2 expression; episialin depolarized expression correlated with poor prognosis, especially in SCC cases; episialin expression defined a group of patients with poor prognosis in the node-positive category and, in multivariate analysis, episialin was the most significant independent prognostic factor followed by nodal stage; despite the fact that their activity on the cadherin cell-cell adhesion system is similar, expression of episialin and c-erbB oncoproteins appears to be activated within different pathogenic pathways (Guddo F, etal, J Clin Pathol, Sep 1998;51(9):667-71)

p16 (CDKN2/MTS-1/INK4A)

The p16 (CDKN2/MTS-1/INK4A) gene is frequently inactivated by DNA methylation in lung cancer; it has been theorized that a possible link may exist between hypermethylation of p16INK4A CpG islands during cigarette smoke exposure and the development of nsclc; when the methylation status of exon 1a of the p16 INK4A gene was determined in 76 consecutive nsclc patients undergoing surgical resection, no association was found in univariate analysis between methylation of p16INK4A CpG islands and smoking, tumor histology, pathologic stage, or the presence of k-ras codon 12 mutation. However, when data was analyzed by multivariate logistic regression stratified by histologic subtype, an association (OR=3.8) was detected between p16INK4A methylation and years smoked only in SCC (Kim D-H, etal, AACR00, Abs. 3165:496)

p53

Mutations in loop-sheet-helix motif of p53 are associated with a poor prognosis in nsclc; such mutations correlated with overexpression of bax, which may result in a poor prognosis in patients with bcl-2+ tumors; when 203 nsclc tissue samples were analyzed, 79 carcinomas (38.9%) were bcl-2+, 46 (71.9%) were bax+, and 72 (35.5%) had missense mutations of p53; there was no significant difference of bcl-2 expression in relation to p53 mutations; however, bax expression was significantly higher in tumors with mutations in loop-sheet-helix motif of p53 than in tumors with wild-type (wt) p53 while bax expression was significantly lower in tumors with mutations of L3-loop of p53, and those with structural mutations of p53 than in those with wt p53; bcl-2/bax ratio status was a significant prognostic factor in nsclc but there was no survival difference in relation to bcl-2 status in a group of mutations of loop-sheet-helix motif of p53; in addition, among patients with bcl-2+ tumors, the 5-year survival of patients with a p53 mutation in loop-sheet-helix motif was significantly lower than that with wt p53, or p53 with structural mutation (Huang C, etal, *ibid*)

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Platelet-derived endothelial cell growth factor (PD-ECGF)/thymidine phosphorylase (TP)

PD-ECGF/TP was detected in the extracts from 137/139 specimens of primary lung cancer at concentrations ranging from 2.0 to 169.5 U/mg protein; PD-ECGF/TP concentrations in patients with adenocarcinoma (n=73) and SCC (n=49) were 30.7+/-22.9 U/mg protein (range=7.6 to 169.5 U/mg protein) and 32.0+/-19.8 U/mg protein (range=8.0 to 84.4 U/mg protein), respectively; there was no significant difference in PD-ECGF/TP concentration between these two types of nsclc, but a >8-fold lower mean concentration of PD-ECGF/TP was found in tissue extracts from sclc (n=17; 3.65+/-2.01 U/mg protein, ranging from undetectable to 6.1 U/mg protein) than in those from adenocarcinoma or SCC; this considerable difference in PD-ECGF/TP concentrations between sclc and nsclc may indicate that, in these two types of lung cancer, angiogenesis involves alternative pathways, and inhibitors of PD-ECGF/TP, currently in preclinical evaluation may not be effective against sclc (Yamashita J, etal, Chest, Jul 1999;116(1):206-11)

Protein gene product 9.5 (PGP9.5)

Using the serial analysis of gene expression method (SAGE; Genzyme Oncology), it was observed that the PGP9.5 transcript was highly expressed in 98 resected primary nsclc, and lung cancer cell lines, but was not detectable in normal lung tissue; PGP9.5 reactivity in normal lung followed a pattern compatible with K-cells of the diffuse neuroendocrine system; however, PGP9.5 was present in both sclc and nsclc cell lines (22/24) independent of neuronal differentiation; in primary nsclc, 54% (53/98) of cases stained positively for PGP9.5, and PGP9.5 expression was strongly associated with cancer stage; it was present in 44% (29/66) of Stage I and in 75% (24/32) of Stage II and IIIa nsclc (Hibi K, etal, Am J Pathol, Sep 1999;155(3):711-5)

Protein phosphatase 2A (PP2A) and PPP2R1B gene on chromosome 11q22-24

PP2A is one of the major cellular serine-threonine phosphatases; PPP2R1B gene that encodes a regulatory subunit of PP2A, is genetically altered in several types of human cancers, indicating that aberrations of intracellular signaling pathways via PPs are involved in human carcinogenesis; in its normal state, PPP2R1B regulates phosphate that binds to proteins that control tumorigenesis suggesting that PPP2R1B gene functions as a tumor suppressor; when defective, it allows phosphate levels to rise uncontrollably leading to tumor cell proliferation; genetic mutations of PPP2R1B may be heritable, and confer susceptibility to such carcinogens as cigarette smoke; the PP2R1B gene product may suppress tumor development through its role in cell cycle regulation and cellular growth control; PPP2R1B alterations have been identified in 5/33 (15%) of lung cancer cases and in 4/70 (6%) lung tumor-derived cell lines (Wang SS, etal, Science, 9 Oct 1998;282(5387):284-7)

Protein tyrosine phosphatase gamma (PTPγ)

Using a PTPγ-specific MAb (γTL1; IgM isotype), it was demonstrated that the expression of PTPγ is severely reduced (>50%) in lung tumors; PTPγ was expressed in more than 90% of both normal human tissue samples, and in the non-tumor cells of carcinoma samples, but was absent in 28% of lung tumor samples, and in 50% of lung adenocarcinoma samples, while expression was weak and heterogeneous in 71% of SCC; PTPγ was not suppressed in normal cells located between lung carcinoma cells (van Niekerk CC and Poels LG Cancer Lett, 22 Mar 1999;137(1):61-73)

Ribonucleotide reductase subunit M1 (RRM1) located on chromosome 11p15.5, LOH11A region

LOH11A is lost in 75% of lung cancers; when this 650-kb region was mapped, among 22 putative genes, RRM1 was singled out as the most likely candidate gene with metastasis suppressor function because of its location at the site of maximal allele loss, and previous functional studies; the malignant phenotype, in this case, resulted from a relative rather than a complete loss of PRM1 function (Bepler G, etal, Genomics, 15 Jan 1999 Jan;55(2):164-75). RRM1, that is composed of 19 exons, encodes the large subunit (M1) of ribonucleotide reductase, the heterodimeric enzyme that catalyzes the rate-limiting step in DNA synthesis; LOH was detected in 48% (15/31) of informative lung tumor specimens; no mutations were revealed in 12 pairs of normal and tumor DNA samples of the 19 RRM1 exons (Pitterle DM, etal, Mamm Genome, Sep 1999;10(9):916-22)

Survivin

Expression of the survivin gene was studied in 83 tumor samples (42 SCC, 37 adenocarcinomas and 4 large-cell carcinomas) from patients with up to Stage IIIa nsclc who had undergone radical surgery, and in neighboring normal lung tissue; RT-PCR identified survivin gene transcript in 71 (85.5%) of the tumor samples, but in only 10 (12%) of the paired normal lung samples; there was no relationship between survivin gene expression and histologic subtype or age, sex, cigarette smoking history, tumor differentiation, tumor size, or the presence of mediastinal lymph node metastases in surgical specimens; 12/83 patients without survivin expression experienced a significantly better overall survival than 71 patients with survivin expression (relative risk=2.1) (Rosell R, etal, ASCO99, Abs.1901:493a)

Tissue factor pathway inhibitor-2 (TFPI-2)

Expression of TFPI-2 is decreased significantly during tumor progression in various cancers; subsequent to the stable transfection of the human lung cancer cell line (A549) which produces high levels of TFPI-2, with a vector capable of expressing an antisense transcript complementary to the full length TFPI-2 mRNA, parental cells, and stably transfected clones, were analyzed for TFPI-2 transcripts and for TFPI-2 protein levels; TFPI-2 mRNA/protein levels were significantly reduced in antisense clones compared to parental and vector controls; when the invasive potential of the parental cells, and stably transfected antisense clones, was measured *in vitro*, the antisense clones exhibited a significantly higher level of invasion than the controls; this data suggest that TFPI-2 expression is critical for the invasiveness/metastasis of lung cancer cell lines *in vitro* (Lakka SS, etal, AACR00, Abs. 3849:604)

Transferrin receptor (Tfr) located on chromosome 3q26

Tfr is transcribed in lung SCC, and may be involved in its pathogenesis (Racz A, etal, Eur J Cancer, Apr 1999;35(4):641-6)

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Tumor inhibitor of metalloproteinase-1 (TIMP1)
When TIMP1 RNA expression level in 45 cases of nsclc and adjacent normal lung tissue was correlated to clinicopathologic features in resected primary nsclc, it was significantly higher in the adenocarcinoma compared to the SCC subtype, although TIMP1 RNA expression levels were generally heterogeneous in nsclc; TIMP1 RNA levels did not correlate with gender, smoking history, or tumor, node, or TNM stage, but there was a statistically significant survival disadvantage in cases with relatively high TIMP1 RNA expression, suggesting a role for TIMP1 in determining the prognosis of resected nsclc (Fong KM, etal, Clin Cancer Res, Aug 1996;2(8):1369-72)
Tumor necrosis factor-related apoptosis-inducing ligand-receptor 2 (TRAILr2) on chromosome 8p21-22
TRAILr2 is a cell-surface receptor involved in cell death signaling; when the entire coding region and all splice sites of TRAILr2 gene were analyzed to detect somatic mutations in a series of 104 nsclc samples, 11 tumors (10.6%) were found to have TRAILr2 gene mutations in the death domain known to be involved in the transduction of an apoptotic signal; somatic mutation of TRAILr2 may play a role in the pathogenesis of some nsclc, and TRAIL r2 gene is one of the genes relevant to the frequent loss of chromosome 8p21-22 in nsclc (Lee SH, Cancer Res, 15 Nov 1999;59(22):5683-6)
Vascular endothelial growth factor (VEGF) on chromosome 6p21.3
Among 120 cases of early-stage nsclc (81 SCC and 39 adenocarcinomas), treated with surgery alone (median follow-up=63 months; range=45-74 months), VEGF expression was positively associated with high vascular grade (microvessel score of >75 per x 250 field), although about half of low vascular grade cases also expressed VEGF; wt p53 expression was inversely associated with VEGF expression, suggesting that wt p53 is involved in the suppression of the VEGF gene; combined analysis of VEGF, wt p53, and microvessel score showed that, although wt p53 loss was associated with VEGF switch-on, p53 protein may not be involved in the regulation of angiogenic events downstream of VEGF expression; there was no significant association of bcl-2 and c-erbB-2 oncoprotein expression, or correlation of T/N stage, grade, Ki67 proliferation index, and extent of necrosis with VEGF expression which was dependent on wt p53 loss and correlated with poor survival, particularly in node-negative cases (Giatromanolaki A, etal, Clin Cancer Res, Dec 1998;4(12):3017-24)

investigations. Results indicated that hnRNP overexpression was more accurate in detecting evidence of neoplasia than routine cytologic examination. hnRNP A2/B1 was overexpressed in 22/23 specimens in which malignant cells were identified cytologically but, among 80 specimens that were reported as cytologically negative, hnRNP was overexpressed in 41 (51.2%); in 29 (36.2%) of these cases a lung neoplasm was diagnosed based on biopsy and/or radiologic findings. An additional 4/41 patients were diagnosed with a lung neoplasm within 8 months after the initial bronchoscopy. Detection of hnRNPA2/B1 in bronchial lavage specimens predicted the presence of a neoplasm with a sensitivity of 96%, and a specificity of 82% (Fielding P, etal, Clin Cancer Res, Dec 1999;5(12):4048-52). All in all more than 6,000 individuals have been screened with this biomarker in ongoing clinical trials in North America, the UK, China, and Japan.

Currently, the Lung Cancer Early Detection Working Group (LCEDWG) is conducting a multicenter trial of hnRNP A2/B1 protein as a biomarker for the early detection of second primary lung cancer, in the USA and Canada, supported by a CRADA funded by the NCI, Moffitt Cancer Center (Tampa, FL), and Bayer Diagnostics (Tarrytown, NY). The sensitivity and specificity of the hnRNP A2/B1 biomarker for later second primary lung cancer were 77% to 82%, and 65% to 81%, respectively. Among the cases identified as positive by immunocytochemistry and image cytometry, 67% developed second primary lung cancer within one year. This diagnostic accuracy exceeds that commonly found in prostate-specific antigen cancer screening tests. One of the objectives of the CRADA is the development of a high-throughput platform of the hnRNP A2/B1 assay.

It also appears that hnRNP B1 alone may be a useful marker for early detection of lung SCC. Overexpression of hnRNP B1 protein was detected in 100% of human lung SCC (Goto Y, etal, Jpn J Cancer Res, Dec 1999;90(12): 1358-63). When hnRNP A2/B1 mRNA and hnRNP B1 mRNA were studied separately, there was evidence that hnRNP B1 mRNA, which is a splicing variant of hnRNP A2 mRNA, was more significantly elevated in lung cancer tissues than hnRNP A2/B1 mRNA. An hnRNP B1-specific polyclonal antibody, selectively identified hnRNP B1 protein as a M(r) 37,000 protein in the nuclei of human cancer cells, and in SCC in particular, but not in those normal adjacent lung epithelial cells (Sueoka E, etal, Cancer Res, 1 Apr 1999;59(7):1404-7).

Homeobox (HOX) genes detectable in lung cancer cell lines, and patient specimens, may serve as a screening tool. Investigators at Karmanos Cancer Institute (Detroit, MI) and the NCI discovered that 3 HOX genes, HOX A7, C5 and D13 were frequently transcribed in patient tumor samples, and were also found in sputum, lavage, and peripheral circulation of some cancer patients. Loss of tumorigenicity was observed when a normal expression pattern was re-established by fusion of cancer cells with non-HOX-expressing airway epithelial cells. Normal lung parenchyma from patients with lung cancer, and circulating cells from healthy volunteers, did not show transcription of the markers, leading to the conclusion that HOX genes may serve as a reliable lung cancer screening tool. Furthermore, these genes are not only detectable in tumor tissue, but also in lavage and sputum. Expression of these genes may implicate elements further downstream involved in later stages of oncogenesis (Fugaro JM, etal, AACR00, Abs. 3813:599).

MAb 28K29 was created from a hybridoma obtained by *in vitro* stimulation of regional lymph node lymphocytes from lung adenocarcinoma patients, and electrofusion of the stimulated cells with murine or human-mouse myeloma cells reactive to lung cancer cells. MAb 28K29 (M(r)~600,000) recognized cell-surface antigens of a lung adenocarcinoma cell line (A549), and demonstrated a significant complement-dependent cytotoxicity to A549 (Yoshinari K, etal, Br J Cancer, Aug 1996;74(3):359-67). MAb28K29 was shown to be highly reactive with all grades of differentiation of lung adenocarcinomas, and with large cell carcinoma. MAb 28K29 reacted with an antigen localized in the membrane and cytoplasm of lung cancer cells. Investigators at Asahi Chemical Industry (Shizuoka, Japan), found that MAb 28K29 reacted with 83% (5/6) of well-differentiated, 79% (22/28) of moderately-differentiated, and 67% (4/6) of poorly-differentiated lung adenocarcinoma specimens taken from 100 patients with lung cancer. MAb 28K29 also reacted with 35% (14/40) of SCC, 70% (7/10) of large-cell carcinoma, and 20% (2/10) of sclc specimens. Expression of 28K29 antigen may prove a useful marker to detect both large-cell carcinoma, and adenocarcinoma of the lung (Yoshinari K, etal, Lung Cancer, Aug 1999;25(2):95-103).

Micrometastases detection, by identifying the presence of malignant cells in bone marrow, may also provide a means of improving the management of nsclc. In September 1999, Nexell Therapeutics (Irvine, CA) began work on a 2-year, multicenter study of a MAb-based tumor enrichment column (TEC) for the detection of breast, prostate, and lung cancer micrometastases in bone marrow and blood products. Funding for the project was provided by a $535,000 NIH SBIR grant.

In preclinical studies, a TEC-enhanced assay detected one tumor cell in 100,000,000 blood cells, a level that surpasses even the most sensitive molecular assays, including PCR. As a result, this method identified more marrow or peripheral blood samples contaminated with tumor than anticipated. Results of a phase I clinical trial, presented on September 7 at the 10th Anniversary Symposium on Autologous Peripheral Blood Stem Cell Transplantation in Mulhouse, France, indicated that use of the TEC system increased the detection sensitivity of immunocytochemical staining, using Nexell's Cytonex ICC staining kit, for minimal residual disease in autologous hematopoietic grafts from breast cancer patients.

Microsatellite alterations in plasma DNA of patients may provide a means for the early diagnosis of nsclc. Investigators at the Istituto Nazionale Tumori (Milan, Italy) looked for microsatellite instability (allele shift), and loss of heterozygosity (LOH), in plasma DNA of 87 Stage I-III nsclc patients, and 14 controls, by combining two markers with a high rate of instability (D21S1245), and LOH (FHIT locus). A microsatellite alteration was observed in 49/87 (56%) nsclc tumors, and in 35/87 (40%) plasma samples; 30/49 (61%) cases showing tumor alterations also displayed a change in plasma DNA and, in addition, 5 patients displayed alterations in plasma samples only. No genetic changes were found in the plasma of controls. There was no association between the frequency of microsatellite alterations in plasma and tumor stage or histology. Plasma DNA abnormalities were detectable in 43% of Stage I cases, and in 45% of tumors up to 2 cm in maximum diameter, indicating that early tumor detection is possible by this *in vitro* approach (Sozzi G, etal, Clin Cancer Res, Oct 1999; 5(10):2689-92).

Telomerase is a specific marker for malignant lung disease, and may be used in conjunction with cytology in the diagnosis of certain lung cancer cases. Investigators at Roy Castle International Centre for Lung Cancer Research, using the telomeric repeat amplification protocol (TRAP) assay, detected telomerase activity in bronchial lavage samples in 17/36 (47%) individuals being evaluated for lung cancer. In particular, 16/23 (70%) bronchial lavage specimens exhibited detectable telomerase activity, compared to only 1/13 (8%) specimen obtained from controls. Moreover, 9/10 (90%) bronchial lavage specimens, that were cytologically positive for lung cancer, were also positive for telomerase activity, while 7/13 (54%) cytologically negative specimens also showed detectable telomerase activity. Detection of telomerase activity, combined with cytology, identified 17/23 (74%) lung cancer cases whereas cytology alone identified 10/23 (43%) such cases (Xinarianos G, etal, Lung Cancer, Apr 2000;28(1):37-42).

Using PCR based on a TRAP assay, telomerase activity was detected in 85/103 (82.5%) nsclc specimens but in none of paired normal lung tissue. More cases of positive telomerase activity were observed in advanced disease, and in poorly differentiated tumors. Telomerase activity did not correlate with mean age at surgery, sex, smoking history, histologic type, and size of tumor extension. Patients with telomerase-positive tumors survived for a significantly shorter period than those with telomerase-negative tumors. According to a multivariate analysis, telomerase activity was identified as an independent prognostic marker (RR=8.62) in nsclc (Taga S, etal, Ann Surg, Nov 1999;230(5):715-20).

Telomerase in the blood may also be a harbinger of cancer spread in those who present with localized lung cancer at the time of diagnosis. Using the TRAP assay, investigators were able to consistently measure telomerase activity in the plasma of more than 90% of lung cancer patients, demonstrating that it can be used as a valid indicator of disease status and progression. Lexon (Tulsa, OK) is developing a telomerase-based blood test in the ELISA format for the early detection of lung cancer spread. Exclusive rights to this product, were obtained by an outright purchase of Cancer Diagnostics (CDI; Kyoto, Japan). This test is being developed by its inventor, Dr. W. Edward Highsmith and colleagues, at the University of Maryland (Baltimore, MD).

STAGING

Staging in lung cancer defines the extent of the disease, determines treatment strategies, and stratifies patients into similar therapeutic and prognostic categories by predicting outcome. Accurate staging is extremely important because treatment and prognosis depend largely on disease stage at the time of diagnosis. More accurate staging at the time of initial presentation may avoid inappropriate surgical decisions in individual patients. Also, accurate staging could improve the design of clinical trials.

During staging, an evaluation is made regarding the size and extent of the primary tumor, lymph node involvement, and the presence of distant metastases. The most important question is whether the tumor is resectable. Preresection staging of patients with nsclc is not straightforward, especially in patients with negative mediastinal nodes.

Staging is carried out using both surgical and nonsurgical techniques. Conventional nonsurgical approaches include posteroanterior and lateral chest chest x-rays, CT scans of the chest and upper abdomen, including the liver and adrenal glands, bone scintigraphy and brain CT, or magnetic resonance MR imaging. In addition, whole-body 2-[fluorine 18]fluoro-2-deoxy-D-glucose (FDG) PET is slated to become the single most important nonsurgical noninvasive staging approach with broad applications in the management of lung cancer. Although CT scanning provides exquisite anatomic information, it is less than optimal for determining lymph node status. Over the last several years, CT scanning combined with FDG PET, has significantly improved the accuracy of clinical staging. FDG-PET is being used in the noninvasive evaluation of the primary tumor, nodal involvement, and metastatic disease. However, despite recent advances in radiologic assessment of nsclc, invasive sampling is still often performed for pathologic confirmation (Lau CL and Harpole DH Jr, Semin Surg Oncol, Mar 2000;18(2):116-23).

With regard to the primary tumor (T status), the accuracy of CT or MR to predict the need for extended resections is limited. Similarly, although all noninvasive methods to determine nodal status (N) are valuable, mediastinoscopy has a greater sensitivity and specificity than either CT or MR. The role of routine organ screening for the detection of distant occult metastasis in the asymptomatic patient remains controversial. Ultimately, the prognosis of the resected patient with lung cancer is based on complete intraoperative staging. At present, however, neither of these techniques has been shown to improve the quality of staging or survival (Deslauriers J and Gregoire J, Chest, Apr 2000;117(4 Suppl 1):96S-103S).

Revisions in the International System for Staging Lung Cancer

The international system for staging nsclc was recently revised (Exhibit 2) to group patients with similar prognoses who are candidates for similar treatment options, in more specific subcategories within the TNM system. This revision was based on a database that collected all clinical, surgical-pathologic, and follow-up information on 5,319 patients treated for primary lung cancer, that confirmed the validity of the TNM and stage grouping classification schema (Mountain CF, Chest, Jun 1997;111(6):1710-7). One of the hallmarks of the revised staging system is the designation of anatomic landmarks for 14 hilar, intrapulmonary, and mediastinal lymph node stations, providing for consistent, reproducible, lymph-node mapping (Mountain CF and Dresler CM, Chest, Jun 1997;111(6): 1718-23).

Noninvasive Imaging

Noninvasive imaging has not reached the level of accuracy required in disease staging. For instance, the Radiology Diagnostic Oncology Group reported that the sensitivity and specificity of CT scanning is only 52% and 69%, respectively. Furthermore, MR imaging does not appear to improve the accuracy of staging. Early evaluation of the role of PET suggests that the combination of CT and PET may have greater sensitivity and specificity than CT alone. A report evaluating the staging of 1,400 patients undergoing tumor resection found that clinical staging by radiologic approaches accurately assessed the T stage in 78% of patients and the N stage in only 47% of patients. Errors in clinical staging were equally divided between overstaging and understaging.

CT staging is an important part of pretreatment evaluation but chest CT has a false-positive rate of 30%, and a false-negative rate of 10%. Furthermore CT is superior to MR in accurately measuring tumor size. To determine whether contrast material-enhanced helical CT of the thorax and upper abdomen changes the tumor stage and management compared with nonenhanced helical CT in patients with newly diagnosed lung cancer, during a 15 month period, patients underwent nonenhanced thoracic helical CT from the lung apices through the adrenal glands, and then contrast-enhanced thoracic helical CT from the lung apices through the entire liver. Each study was read independently to determine thoracic radiologic stage. Among 96 patients with a final pathologic diagnosis of lung cancer, there was agreement in stage between the nonenhanced and contrast-enhanced CT in 92 (95.8%). In 3 patients, tumor stage, based on nonenhanced CT, increased at contrast-enhanced CT, from IA to IIA (n = 1), IIb to IV (n = 1), and IIIb to IV (n = 1) and, in one patient, stage decreased from IIIb to IIb. Therefore, contrast-enhanced thoracic CT through the liver for staging lung cancer rarely changes the tumor stage determined with nonenhanced CT through the adrenal glands, and does not substantially influence management decisions (Patz EF Jr, Radiology, Jul 1999;212(1):56-60).

FDG PET imaging provides physiologic and metabolic information, and may characterize lesions that cannot be defined by CT, accurately stages lung cancer, and yields prognostic information. The sensitivity of FDG PET is about 95%, and its specificity about 85%, slightly less than

its sensitivity because some inflammatory processes, such as active granulomatous infections, avidly accumulate FDG. This modality's specificity is particularly important because its high negative predictive value prevents unnecessary biopsies by permitting follow-up by radiography alone. Several studies have documented increased accuracy with PET, compared with CT, in the evaluation of hilar and mediastinal lymph-node status in nsclc.

Whole-body FDG PET, appears to be more accurate than thoracic CT, bone scintigraphy, and brain CT or MR imaging in staging bronchogenic carcinoma. Whole-body PET detects metastatic disease that is not picked up by conventional imaging, and designates some of the anatomic abnormalities detected by CT as benign lesions. Management changes have been reported in up to 41% of patients on the basis of the results of whole-body PET studies (Coleman RE, J Nucl Med, May 1999;40(5):814-20).

To investigate the role of FDG PET in staging of nsclc, 100 patients with newly diagnosed bronchogenic carcinoma, underwent whole-body FDG PET, and chest CT, within 20 months at Duke University Medical Center (Durham, NC); 90/100 also underwent radionuclide bone scintigraphy, and 70/100 brain CT, or MR imaging. Each examination was completed within one month. FDG PET and conventional imaging were used independently to assign a radiologic stage which was then compared with the pathologic stage. PET staging was accurate in 83% of cases while staging with conventional imaging in 65%. Staging with mediastinal lymph nodes matched that with PET in 67 (85%) cases, and with CT in 46 (58%). Metastases not detected with conventional imaging, were found by PET in 9% of patients whereas 10% of cases, classified as metastatic by conventional imaging, were correctly deemed negative with PET (Marom EM, etal, Radiology, Sep 1999; 212(3):803-9).

MR scanning when properly conducted and interpreted, is comparable to more invasive methods such as cervical mediastinoscopy, or VATS. Pathologic results of 543 lymph nodes removed during 164 radical procedures at the University Medical School of Pecs, in Hungary, were compared with expected findings based on preoperative T2-weighted MR imaging. Specificity of MR images was 95.5% for individual lymph nodes, and 88.1% for TNM staging, while sensitivity was 89.4% for lymph nodes and 94.6% for TNM staging, and accuracy was 84.7% (Molnar TF, etal, Acta Chir Hung 1999;38(1):95-9). Actually MR may be more useful than CT in examining small nodules near hilar vessels, and is better at imaging chest wall involvement because of its higher contrast resolution, and multiplanar capabilities, resulting in an accuracy of 94%.

Surgical Staging

Surgical staging involves histologic assessment of the primary tumor, and potential sites of metastases. Currently, the standard for surgical staging is cervical mediastinotomy. Other minimally invasive surgical procedures include scalene lymph node biopsy, bronchoscopy with transbronchial biopsy, anterior mediastinoscopy, and VATS (Matin TA and Goldberg M, Oncology (Huntingt), May 1999;13(5):679-85; discussion 685, 689, 693).

Mediastinotomy/mediastinoscopy is currently the standard for surgical staging when accurate evaluation of the nodal status is needed to determine therapy. This procedure is standard when hilar lymph nodes, viewed by x-ray or CT, are enlarged implying tumor infiltration. Most common is cervical mediastinotomy but anterior mediastinoscopy is also used as a surgical staging approach. Cervical mediastinoscopy is performed via a small cervical incision above the sternal notch. Although bilateral examination of the mediastinum is possible, cervical mediastinoscopy does not permit access to lymph nodes in the periaortic area, or to those located in the anterior mediastinum. These latter areas are accessible with anterior mediastinotomy which is carried out by a limited anterior thoracotomy. Although this approach permits investigation of the pulmonary artery and other hilar structures, it does not provide access to the ipsilateral mediastinum.

Although nodal dissection for staging is an important component of intrathoracic staging of disease in patients scheduled to undergo thoracotomy for lung cancer, such dissection is not performed routinely in all cases because it is nearly impossible to identify a clinical or pathologic subset of patients with a negligible incidence of N2 disease so that findings with nodal dissection would categorically assess nodal status. (Graham AN, etal, J Thorac Cardiovasc Surg, Feb 1999;117(2):246-51).

Thoracoscopy and video-assisted thoracoscopy (VATS) may be more accurate staging procedures. When the results of radiologic, thoracoscopic, and pathologic staging in nsclc patients with negative mediastinoscopy were compared, thoracoscopic staging was more accurate than CT staging. For instance, errors in thoracoscopic staging resulted in no inappropriate operations but errors in CT staging would have resulted in operations unlikely to help the patients, or would have inappropriately excluded patients from surgery (Roberts JR, etal, Ann Thorac Surg, Oct 1999;68(4):1154-8).

Intra-operative sentinel lymph node mapping, a procedure used in melanoma and breast cancer, may also be useful in identifying involved lymph node stations, assessing drainage patterns of lung tumors, and in surveying the chest for residual nodal tissue which may harbor metastatic disease, in order to improve staging, and help identify subsets of patients requiring more aggressive interventions. Investigators at the Kellogg Cancer Center (Evanston, IL) conducted a phase I clinical trial of intraoperative sentinel thoracic lymph-node mapping with technetium-99 (Tc-99)-labeled colloid in patients with Stage I-II primary nsclc at the time of thoracotomy. The primary tumor was injected with a total dose of 2 mCi in a

four quadrant injection pattern of 0.5 mCi, suspended in 0.5 cc per injection. Scintographic readings of both the primary tumor, and draining lymphatics, were obtained with a hand-held gamma probe counter. Resected tissue was then examined histologically. Radioisotope migration through regional lymphatics was detected in 7/9 enrolled patients. Mean migration time from injection was 43 minutes. Two patients underwent immediate wedge resections prior to migration. Sentinel nodes were identified in 5/7 evaluable patients (3 with positive histology). In two patients with detectable radioisotope migration, histologically positive intralobar nodes within the resected specimen were clinically indistinguishable from the primary tumor. In one patient, residual activity in an anterior tracheal level 3 station required further resection before closure. No unexpected toxicities or complications occurred indicating that this approach is feasible and safe (Liptay M, etal, ASCO99, Abs. 1871:485a).

Molecular Substaging

A molecular staging protocol using reliable markers is of importance in assessing the prognosis of patients with nsclc, and in selecting appropriate postsurgical treatment. Among the numerous combinations and permutations of markers that have been investigated in this area, a selected few are described below.

Low MRP-1 and KAI1 expression in nsclc may be associated with poor prognosis, and evaluation of MRP-1 and KAI1 expression may identify node-negative lung cancer patients who are at high risk for early disease recurrence, and thus need intensive adjuvant therapy (Adachi M, J Clin Oncol, Apr 1998;16(4):1397-406). Tumor tissues from 187 nsclc patients were analyzed to establish the presence of mutations of p53 at exons 5-8, mutations of K-ras at exon 1, MRP-1/CD9 gene, and KA11/CD82 gene expression, which have been postulated to be metastasis suppressor genes. Nodal status, MRP-1/CD9 and K-ras status were significant factors for prognosis. Based on these results, patients were classified into three groups according to their MRP-1/ CD9 and K-ras status that significantly correlated with tumor status and pathologic stage:

Patient Group	Marker	Overall Survival Rate (%)
Group A	Wild K-ras tumors that are also MRP-1/CD9 positive	59.6
	As above, but node-negative*	75.8
Group B	Tumors with reduced MRP-1/CD9 expression, or tumors with mutant K-ras	27.9
	As above, but node-negative*	34.9
Group C	Mutant K-ras tumors with reduced MRP-1/CD9 expression	20.0
	As above, but node-negative*	0.0

**Based on 110 node-negative nsclc*

Multivariate regression analysis demonstrated that an evaluation for both MRP-1/CD9 expression and K-ras mutations, as well as nodal status, were significantly prognostic in nsclc (Miyake M, etal, Oncogene, 8 Apr 1999;18(14): 2397-404).

Molecular biologic substaging, according to gender and histology, was performed at Duke University Medical Center, on 408 consecutive patients after complete resection for Stage I nsclc, who were follow-up for at least 5 years. A panel of nine molecular markers was chosen for immunohistochemical analysis, including recessive oncogenes p53 and bcl-2, proto-oncogene erbB-2, KI-67 proliferation index, rb oncogene, EGFr, angiogenesis factor VIII, sialyl-Tn antigen (STN), and CD44. Among men, the only molecular marker associated with decreased cancer-specific survival was erbB-2; among women, four markers, p53, rb, CD44, and factor VIII, were involved. Among patients with SCC, the only molecular marker associated with decreased cancer-specific survival was erbB-2; among patients with adenocarcinoma there were three markers, p53, CD44, and angiogenesis factor VIII. Based on multivariable analysis, a hazard ratio of 2.269 was established for female gender and p53+ status, and of 2.266 with female gender and CD44+ and adenocarcinoma (D'Amico TA, etal, Ann Thorac Surg, Mar 2000;69(3):882-6).

All patients with Stage I nsclc, resected at Brigham and Women's Hospital (Boston, MA), between 1984 and 1992, with adequate clinical follow-up, were studied retrospectively to construct a comprehensive multivariate model of cancer recurrence, and to design a molecular pathologic substaging system in Stage I nsclc. Among 244 cases studied (there were 25 noncancer deaths in this group and disease recurred in 80 patients), investigators assessed the importance of three demographic characteristics, surgical extent, 11 pathologic features, and seven molecular factors on cancer-free survival. Significant univariate predictors of cancer recurrence were >60 years-of-age, male sex, wedge resection, adenocarcinoma with mucin, lymphatic invasion, and p53 expression. Multivariate analysis identified nine independent predictors of recurrence, adenocarcinoma with mucin, wedge resection, tumor diameter ≥4 cm, lymphatic invasion, >60 years-of-age, male sex, p53 expression, K-ras codon 12 mutation, and absence of H-ras p21 expression. Multivariate cancer-free survival analysis in the 180 patients who underwent lobectomy or pneumonectomy, led to the elimination of sex and age, which left six independent factors. These findings indicate that lobectomy, or pneumonectomy, should be performed in Stage I nsclc. Using the six independent factors for recurrent disease, a pathologic molecular substaging system is proposed as follows: patients with ≤2 factors are considered Stage Ia, with a 5-year disease-free survival (DFS) rate of 87%; those with 3 factors, Stage Ib, with a 5-year DFS rate of 58%; and those with ≥4 factors, Stage Ic, with a 5-year DFS rate of 21% (Kwiatkowski DJ, etal, J Clin Oncol, Jul 1998;16(7): 2468-77).

In a similar analysis, performed at Duke University, pathologic specimens were collected from 408 consecutive patients after complete resection for Stage I nsclc, with follow-up of at least 5 years. A panel of 10 molecular markers

was chosen for immunohistochemical analysis of the primary tumor on the basis of differing oncogenic mechanisms. Markers included in the analysis were growth regulating proteins, such as EGFr, and erB-b2, that are implicated in local tumor expansion; apoptosis proteins such as p53, and bcl-2; cell cycle regulating proteins such as rb recessive oncogene, and KI-67; angiogenesis factor VIII; and expression of adhesion proteins CD44, sialyl-Tn, blood group A, involved in the development of distant metastases. Multivariable analysis demonstrated significantly elevated hazard ratios for p53 (1.68), factor VIII (1.47); erB-b2 (1.43), CD44 (1.40); and rb (0. 747). Five molecular markers were associated with the risk of recurrence and death, representing independent metastatic pathways such as apoptosis (p53), angiogenesis (factor VII), growth regulation (erB-b2), adhesion (CD44), and cell cycle regulation (rb) (D'Amico TA, etal, J Thorac Cardiovasc Surg, Apr 1999;117(4):736-43).

PROGNOSIS

Prognosis in lung cancer is very important in determining survival outlook, and selecting treatment options. Similar approaches may be applicable in both diagnosis, staging and prognosis, providing a continuum of evaluation in the management of cancer. Diagnosis and staging are applied at first presentation, but prognostic evaluations may be applicable throughout the course of a patient's illness. Therefore, noninvasive modalities such as imaging, and/or *in vitro* tests to assess patient prognosis are particularly attractive options because such procedures may be performed repeatedly with minor patient discomfort throughout the active phase of disease. In addition to their convenience, *in vitro* prognostic tests may also prove to be less inexpensive. However, at this point, although numerous permutations and combinations of markers have been investigated, none has reached the degree of performance required in the clinic.

Currently, predictors of disease-free and overall survival are linked to such obvious factors as stage at diagnosis, age, and performance status. Patient prognosis is particularly important when dealing with early-stage cancer that is usually treated with curative surgery. Considerable efforts are focused on identifying prognostic factors in Stage I disease. For instance, there are few prognostic factors for patients with T1 N0 M0 conventional adenocarcinoma or BAC, despite a 25% to 35% failure rate. To identify prognostic factors related to DFS, investigators at William Beaumont Hospital (Royal Oak, MI) retrospectively studied histologic features of 218 cases of T1 N0 M0 adenocarcinomas. The mean overall follow-up was 5.9 years, and the 5-year DFS was 72%; 148 patients (67.9%) were disease-free, while nonpulmonary metastases developed in 57 (26.1%). Features significantly associated with decreased 5-year DFS were larger tumor size, increasing central fibrosis, most common and highest nuclear grade, lymphatic vascular space invasion, and >50% tumor necrosis. The 5-year metastases-free survival of patients with lymphatic vascular space invasion was 35%. Tumor size of 2 to 3 cm, lymphatic vascular space invasion, highest nuclear grade, and increased central fibrosis, were associated with metastases. The highest OR of 5.4 was associated with lymphatic vascular space invasion. These histologic features can stratify patients with T1 N0 M0 neoplasms, and an increased risk of metastases (Goldstein NS, etal, Am J Clin Pathol; Sep 1999;112(3):391-402).

Serum Markers

Serum markers are particularly attractive as sample is readily accessible, and tests may be repeated as necessary. Serum was acquired prospectively in 132 consecutive patients with clinical Stage I nsclc, before, and one month after, resection. The post-resection pathological stages were Stage I (n=86), Stage II (n=33), Stage III (n=13). Six novel tumor markers of invasion and metastasis, i.e., hepatocyte growth factor (HGF), VEGF, urokinase plasminogen activator (uPA) and receptor (uPAr), E-selectin, and CD44, were measured. Larger tumors (T2 or T3) had higher values before resection, and E-selectin and CD44 values were higher with SCC. Elevated post-resection levels of CD44, uPA, and HGF, were associated, based on univariate and multivariate analyses, with an increased rate of cancer recurrence. This data is being validated in a multicenter trial at Duke University Medical Center (Harpole, DH, etal, AACR00, Abs. 4397:691).

Using a sandwich ELISA developed by IDL Biotech (Sollentuna, Sweden) to detect cytokeratin 8 and 18 (CK-8/18) fragments, investigators at Akademiska Hospital at Uppsala University, in Sweden, tested serum from 69 patients with nsclc. CK-8/18 levels varied between 0.34 ng/ml and 14.2 ng/ml in nsclc cases, compared with a cut-off value of 1.0 ng/ml for controls; elevated levels were detected in 80% of patients with nsclc. A statistically significant diminished survival was observed for CK-8/18 values ≥8.0 ng/ml (Bergqvist M, etal, Anticancer Res, May-Jun 1999;19(3A):1833-6).

The prognostic significance of carcinoembryonic antigen (CEA), NSE, SCC antigen, tissue-type plasminogen activator (tPA), and Cyfra 21.1 serum levels at the time of diagnosis, as well as the predictive ability of these tumor markers with respect to histologic type and pathological stage, were evaluated in 62 patients treated by radical resection at the Universita degli Studi (Milano, Italy); there were 34 adenocarcinomas and 28 SCC, and 32 patients with Stage I, 4 with Stage II and 23 with Stage IIIa disease. SCC serum levels were predictive of histologic type and, as for pathologic stage tPA, and Cyfra 21.1 were found to have moderate predictive value. In this series of patients, at a median follow-up of 55 months after surgery, both tPA and Cyfra 21.1 serum levels at diagnosis were reliable predictors of overall survival, with high values associated with a worse prognosis (Foa P, etal, Int J Biol Markers, Apr-Jun 1999;14(2):92-8).

Elevated preoperative serum concentrations of CEA were found to predict a poor prognosis for lung cancer

independent of other conventional staging parameters such as CT, bronchoscopy, and mediastinoscopy. In a prospective cohort study of 130 consecutive patients, being evaluated for lung cancer, from July 1991 through December 1992, at a Veterans Affairs Medical Center associated with the University of Minnesota School of Medicine (Minneapolis, MN), malignant disease was diagnosed in 111/130. Serum concentrations of CEA were measured before diagnosis, staging, or resection. Among 50/111 patients who underwent resection with curative intent, multivariate analysis indicated that CEA was a significant predictor of survival independent of patient age, pathologic stage, histologic type, and tumor size (Rubins JB, etal, J Thorac Cardiovasc Surg, Sep 1998;116(3):412-6).

Tissue Pathology

Currently, most prognostic evaluations are performed on tumor specimens. An NCI-sponsored clinical trial, being conducted by the Cancer and Leukemia Group B (protocol ID: CLB-9761), has been accruing approximately 400 patients with suspected, or histologically confirmed, untreated nsclc, or Stage I proven, or suspected nsclc (T1 or T2 primary, N1 or N2 lymph nodes <1 cm on CT, or negative mediastinoscopy) to:

- determine whether the presence of occult micrometastases, detected by immunohistochemistry or reverse transcriptase-polymerase chain reaction (RT-PCR) in histologically negative lymph nodes, or bone marrow, is associated with poorer survival among patients with Stage I nsclc
- describe the incidence of occult micrometastases in histologically negative lymph nodes and bone marrow by immunohistochemistry (staining for cytokeratins and MUC1 glycoprotein), and RT-PCR (to detect MUC1 mRNA)
- determine the relationship between tumor size (or T stage) and the presence of occult micrometastases detected by immunohistochemistry and RT-PCR
- determine the relationship between presence of occult micrometastases and DFS
- describe the relationship between the site of occult micrometastases and incidence of recurrence, site of recurrence, and survival

Planned thoracotomy for lobectomy or pneumonectomy, or VATS lobectomy, is acceptable if no preliminary wedge resection of tumor is performed. Samples of bone marrow, primary tumor, and intrathoracic lymph nodes are harvested at the time of thoracotomy and pulmonary resection.

Investigators at the University of Milan, in Italy, evaluated the prognostic significance of DNA content, proliferating cell nuclear antigen labeling index (PCNA-LI), and p53 mutation and apoptosis, in 152 surgically resected nsclc. Results were correlated to histology, stage, and outcome. A considerable variability was found in PCNA indices, ranging from 0% to 33.5%, with a mean value of 7.0%. DNA evaluation showed a prevalence of aneuploid tumors (62%) with a DNA index >1. Overexpression of p53 protein, and apoptotic positivity, were observed in a low percentage of cases (16% and 32%, respectively). On multivariate analysis, only stage and PCNA-LI were of significant prognostic value. PCNA-LI was superior to stage in predicting survival of patients with nsclc. Therefore PCNA-LI immunostaining, that can be applied on a routine basis in formalin-fixed, paraffin-embedded samples of nsclc, may be used to predict patient prognosis (Lavezzi AM, etal, Oncol Rep, Jul-Aug 1999;6(4):819-25).

Angiogenesis evaluation, using microvessel count (MVC) is a new indicator of tumor aggressiveness in patients with nsclc who undergo curative surgery, and should be taken into consideration in selecting patients for adjuvant treatment. In a retrospective study, investigators at Ospedale Santa Maria delle Croci (Ravenna, Italy) evaluated the relationship between tumor angiogenesis and survival, in 76 patients with nsclc, treated with surgery with a curative intention, between 1992 and 1997. Angiogenesis was measured in tumor samples using an anti-CD34 MAb, and quantified in terms of MVC. Other factors considered for analysis included gender, age, stage, histologic type, and KI-67. The median MVC was 41.5. In univariate analysis, among the clinicopathologic parameters examined, MVC was the only one that was significantly associated with DFS (Dazzi C, etal, Lung Cancer, May 1999;24(2):81-8).

When tumor-associated macrophage infiltration (TAM) density, and the density of microvessels, was investigated in specimens from 113 patients with pulmonary adenocarcinoma, there was a significant relationship between TAM density, and microvessel density. There was a difference in survival between patients with tumors with a high TAM density, and those with a low TAM density. Multivariate analysis linked TAM density to survival (Takanami I, etal, Oncology 1999;57(2):138-42).

Genetic Tests

LOH on chromosomes 2q, 9p, 18q, and 22q, frequently occurs in advanced nsclc, but the association of p53 mutations with prognosis is still unclear. The prognostic significance of allelic imbalances (AI) on these chromosomes and of p53 mutations, were investigated in 108 cases of Stage I nsclc. AI on 2q, 9p, 18q, and 22q was detected in 22%, 38%, 29%, and 15% of cases, respectively, whereas p53 was mutated in 41% of Stage I nsclc. AI on 9p and 22q, and p53 mutations were significantly associated with shortened survival. Although gender and smoking history showed more significant associations with prognosis than other clinicopathologic and molecular parameters, independent prognostic significance for AI on 9p was observed in male patients with a positive smoking history. These results indicate that clinical aggressiveness of early-stage nsclc can be partly gauged by the presence of AI on chromosome 9p in cancer cells, and that AI on 9p could be a clinically useful prognostic indicator in early-stage nsclc (Tomizawa Y, etal, Clin Cancer Res, May 1999;5(5):1139-46).

— continued on back page

FDG PET Imaging

Because the amount of radiolabeled FDG uptake reflects the rate of glucose metabolism of nsclc cells, and correlates with proliferation capacity, the standardized uptake value (SUV), a semiquantitative measurement of FDG uptake on a PET scan, may be of prognostic significance. Follow-up data of 125 potentially operable nsclc patients (Stage I/II=65, Stage IIIa=37, and Stage IIIb=23) who had been previously included in three prospective PET protocols, was analyzed by the Leuven Lung Cancer Group, in terms of performance status, maximal tumor diameter, tumor-cell type, SUV, and final staging, for possible association with survival. In univariate analysis, performance status, stage, tumor diameter, tumor-cell type, and SUV >7 correlated with survival. A group dichotomy emerged, with a cut-off SUV of 7 offering the best discriminative value for prognosis, both in the whole 125-patient group, and among 91 patients treated with complete resection. A multivariate analysis identified performance status, stage, and SUV, as prognostic. In the surgical group, patients with a resected tumor <3 cm had an expected 2-year survival of 86%, if the SUV was <7, and 60%, if >7. SUV was >7 in nearly all resected tumors >3 cm with an expected 2-year survival of 43% (Vansteenkiste JF, etal, J Clin Oncol, Oct 1999;17(10):3201-6).

INDEX OF COMPANIES & INSTITUTIONS

Akademiska Hospital	1146	Fujirebio	1136	Kellogg Cancer Center	1144	Russian Academy of Medical Sciences	1135
AMDL	1136	Genzyme Oncology	1140	Klinikum Grosshadern	1135	Truman VA Hospital	1132
Asahi Chemical Industry	1142	Harvard School of Public Health	1130	Ludwig-Maximilians University	1135	Universita degli Studi	1146
Bayer Diagnostics	1141	Hubei Medical University	1136	Lexon	1142	University of Maryland	1142
Brigham and Women's Hospital	1145	Hyseq	1138	M. D. Anderson Cancer Center	1137, 1139	University Medical School of Pecs	1144
Cancer Diagnostics	1142	IDL Biotech	1146	Moffitt Cancer Center	1141	University of Milan	1147
Cross Cancer Institute	1136	Imatron	1135	National Cancer Institute (NCI)	1130, 1141	University of Minnesota School of Medicine	1147
Duke University Medical Center	1144, 1145	INSERM	1130	National Institutes of Health	1142	University of Missouri	1132
Ellis Fischel Cancer Center	1132	International Agency for Research on Cancer	1130	National Naval Medical Center	1133	University of Pittsburgh Cancer Institute	1130
Finnish Institute of Occupational Health	1130	Institute of Pathology, Berlin	1135	Nexell Therapeutics	1142	Uppsala University	1146
Foundation for Blood Research	1133	Istituto Nazionale Tumori	1142	Ospedale Santa Maria delle Croci	1147	William Beaumont Hospital	1146
		Karmanos Cancer Institute	1141	Roche Diagnostics	1136	Yale University School of Medicine	1133
				Roy Castle International Centre for Lung Cancer Research	1136, 1142		

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