J Med Sci 2007;27(1):001-008
http://jms.ndmctsgh.edu.tw/2701001.pdf

Molecular Biologic Staging and Selection of Therapy for Non-Small Cell Lung Cancer

Thomas A. D'Amico*

Division of Cardiovascular and Thoracic Surgery, Department of Surgery, Duke University Medical Center

The optimal staging system achieves accurate assessment of extent of disease, effective prognostic stratification, and selection of appropriate therapy. The staging system for non-small cell lung cancer (NSCLC) provides a framework for the assessment of prognosis and the assignment of therapy for all patients with a new diagnosis of lung cancer, the most common cause of death by malignancy[1]. The most recent revision of the lung cancer staging system, which considers the size and location of the primary tumor (T), the involvement of regional lymph nodes (N), and the presence of distant metastases (M), is based on the analysis of a collected database representing all clinical, surgical-pathologic, and follow-up information for 5,319 patients treated for primary lung cancer[2]. Similar results have been reported among a population of 6,670 patients treated in Japan[3].

The power of these large databases in predicting prognosis is self-evident. Nevertheless, there is an inherent inaccuracy of this staging process. According to the TNM system, the predicted 5-year survival after complete resection for T1N0M0 NSCLC (stage IA) is only 67%[2]. Therefore, 33% of patients with stage IA NSCLC are incorrectly staged at presentation. Even with optimal therapy, these patients will succumb to their disease, predominately from the development of metastatic disease not detected at the time of diagnosis and initial therapy, despite the use of standard staging procedures[4]. Similarly, a significant fraction of all patients with Stage Ib or II disease are incorrectly staged, resulting in inaccurate assessment of extent of disease, prognostic stratification, and selection of therapy. Currently, adjuvant chemotherapy has been established as beneficial for selected patients with after complete resection[5-7]; however, the majority of patients will not benefit, from its administration: substantial fractions will die despite chemotherapy or would have survived even without chemotherapy.

Molecular biologic staging refers to the assessment tumor markers associated with various oncogenic mechanisms in order to improve the risk stratification provided by conventional TNM staging. Biologic staging may target oncogenes, oncogenic protein products, growth factors, or receptors. The biologic techniques utilized include analysis of DNA, RNA, or protein products. Molecular biologic staging may potentially be applied to the primary tumor, lymph nodes, bone marrow, or serum, in order to establish the diagnosis of malignancy at earlier stage, to assess prognosis, to detect occult metastases, to select therapy, and to predict chemotherapy sensitivity or resistance.

The purpose of the assessment of prognostic markers in the primary tumor is to identify patients, or groups of patients, with early stage disease, whose risk of recurrence is sufficiently high enough to justify adjuvant therapy. In addition, the assessment of the primary tumor may also enable more accurate selection of adjuvant therapy, either cytotoxic chemotherapy or targeted therapy. Assessment of lymph nodes may allow identification of micrometastatic disease: occult metastases not identified on routine pathologic examination. Correct assessment of micrometastatic lymph node involvement improves assessment of extent of disease, prognostic stratification, and choice of adjuvant therapy[8]. Assessment of bone marrow and serum may identify evidence of occult distant metastatic disease (Stage IV). Identification of these patients would prevent unnecessary surgical resection and allow patients to receive systemic therapy sooner.

Key words: non-small cell lung cancer (NSCLC); p53; angiogenesis factor VIII; erbB-2; CD-44; and rb; epidermal growth factor receptor (EGFR)

Received: July 24, 2006; Accepted: August 22, 2006
*Corresponding author: Thomas A. D'Amico, Division of Cardiovascular and Thoracic Surgery, Department of Surgery, Duke University Medical Center, Box 3496, Durham, North Carolina 27710, United States. Tel:+1-919-684-4891; Fax:+1-919-684-8508; E-mail:damic001@mc.duke.edu Communicated by Ching Tzao, Division of Thoracic Surgery, Tri-Service General Hospital, Taipei, Taiwan, Republic of China

MOLECULAR STAGING OF THE PRIMARY TUMOR

Molecular biologic substaging, the use of molecular markers as a strategy of risk stratification has been validated in a retrospective studies[9-16] and is under evaluation prospectively. Assessment of the primary tumor with molecular techniques may improve the prognostic stratifica-

tion of patients with NSCLC by predicting which patients are most likely to recur after surgical resection. In addition, the profile of the primary tumor may be used to assess the sensitivity to selected adjuvant therapy.

Characterization of the primary tumor may be made using various molecular markers. The use of a panel of markers may improve the effectiveness of this approach, as expression of individual oncogenic markers is low in NSCLC: p53 and the epidermal growth factor receptor (EGFr) are expressed in approximately 43% and 52% of tumors, respectively[11]. Studies that evaluate molecular prognostic variables must be limited to early stage disease; the inclusion of patients with advanced stage disease dilutes the potential prognostic value of the markers, since this subgroup of patients will have a dismal prognosis, regardless of marker status. In one study of 408 stage I patients who underwent complete resection and no adjuvant therapy, multivariable analysis demonstrated significantly elevated risk for the following molecular markers (hazard ratio; p Value): p53 (1.68; 0.004); angiogenesis factor VIII (1.47; 0.033); erbB-2 (1.43; 0.044); CD-44 (1.40; 0.050); and rb (0.747; 0.084). Each of these factors was improved the stratification independently, and as a composite, molecular substaging identified groups of patients with 5-year survival ranging from 37% (5 negative prognostic markers) to 80% (1 negative prognostic marker). The identification of these factors also establishes potential therapeutic strategies, such as blockade of the erbB-2 receptor in patients with overexpression of erbB-2, the administration of normal p53 in patients with p53 mutations, or antiangiogenic therapy in patients with high angiogenesis factor VIII.

Many molecular markers have been found to improve prognostic stratification of patients with early stage NSCLC after complete resection. The effectiveness of chemotherapy for patients with stage IB NSCLC after complete resection has been demonstrated in a Cancer and Leukemia Group B (CALGB) protocol, CALGB 9633[6]. In this study, patients with completely resected stage IB NSCLC were randomized to receive postoperative chemotherapy (carboplatin and paclitaxel) or observation; all patients in the study will have their tumor analyzed for a panel of molecular markers, in order to determine the prognostic significance of the markers with respect to chemotherapy. Several markers represent a potential avenue for treatment, based on the particular oncogenic mechanism[9-17].

SPECIFIC MECHANISMS FOR THERAPEUTIC INTERVENTION

The expression of specific molecular markers may be used to identify specific oncogenic pathways, which may be used to characterize treatment sensitivity or resistance. In one study, the expression of a panel potential molecular markers of chemoresistance were prospectively evaluated in a population of patients with pathology-confirmed stage III NSCLC in order to determine the prognostic value of each marker in relation to response to chemotherapy or survival[16]. Immunohistochemical staining was performed on histologically positive mediastinal nodal specimens obtained from 59 patients without evidence of distant metastatic disease treated with navelbine-based chemotherapy and external beam radiation therapy between 1996 and 2001. Included were markers for apoptosis (p53, bcl-2), drug efflux/degradation (MDR, GST-), growth factors (EGFr, erbB-2), and mismatch repair (hMLH1, hMSH2). After chemotherapy, patients underwent radiologic evaluation for response measured by standard criteria. Multivariable analysis of marker expression associated overexpression of p53 and low expression of hMSH2 with poor treatment response and cancer death. In addition, there was a significant difference in median survival for patients that expressed none (>60 months), one (18 months), or two (8 months) of the negative prognostic markers ($p< 0.003$)[16].

While numerous markers and pathways have been demonstrated to improve prognostic stratification, therapeutic intervention targeting these pathways is more limited. Targeted therapy is proposed as strategy to deliver mechanistically-specific therapy, with a side effect profile that is superior to cytotoxic chemotherapy. Some of the pathways which are amenable to targeted therapy are reviewed.

Proto-oncogenes *er*bB-1 and erbB-2 (*HER*-2/*neu*)

The proto-oncogene c-erbB-1 encodes for epidermal growth factor receptor (EGFr), a tyrosine kinase-type membrane receptor. Ligand binding to EGFr results in receptor dimerization, autophosphorylation, activation of cytoplasmic proteins, and eventually DNA synthesis[17]. Mutations in erbB-1 can result in constitutive activation of EGFr despite the absence of ligand with the result being uncontrolled tumor growth. In NSCLC elevated levels of EGFr have been shown to be present compared to normal lung tissue. ErbB-2 (also known as *HER*-2/*neu*) shares extensive homology (80%) with erbB-1 and encodes for a transmembrane tyrosine kinase receptor (p185neu) that also functions as growth factor receptor. Kern and colleagues found 10 of 29 patients with adenocarcinoma overexpressed p185neu, and this overexpression was associated with decreased survival[18].

The class of EGFR-targeted therapies contains several agents in various stages of development. EGFR expression is also associated with resistance to chemotherapy and radiotherapy. Two general approaches have been pursued to modify EGFR activity: monoclonal antibodies directed at EGFR or its ligand (EGF) and small molecule inhibitors of the EGFR tyrosine kinase. Both approaches inactivate the EGFR pathway and inhibit tumor activity[19] .

Two small molecule inhibitors in particular have been well studied: gefitinib and erlotinib. Of the EGFR-targeted agents, gefitinib is approved for the treatment of NSCLC; erlotinib is currently under FDA review for an indication in NSCLC. Initial studies of gefitinib demonstrated favorable tolerability and antitumor activity, and the FDA granted an indication for this agent as monotherapy in advanced NSCLC after failure of both platinum-based and docetaxel chemotherapies. Two large-scale clinical trials (INTACT-1[20] and INTACT-2[21]) evaluated the use of gefitinib in combination therapy compared to chemotherapy alone. In these 2 studies, there were no significant differences between groups in median survival.

Erlotinib was evaluated in combination with other chemotherapy agents in 2 recent studies[22,23]. Herbst et al compared the combination of erlotinib plus carboplatin—paclitaxel with placebo plus carboplatin—paclitaxel[22]. A total of 1,059 patients who had not received previous chemotherapy were enrolled. There were no statistical differences between groups in the primary outcome measure of overall survival (10.8 months erlotinib vs 10.6 months placebo; P=0.95). The second trial followed a similar design, but used cisplatin and gemcitabine rather than carboplatin and paclitaxel. The 1,172 patients had not previously received chemotherapy. Again, there were no statistical differences between groups in overall survival or time to progression[23]. In another study, the efficacy of erlotinib was evaluated following the failure of first- or second-line chemotherapy[24]. In this trial, 731 patients were randomized to receive erlotinib or placebo. The erlotinib group showed statistically significant improvements in overall survival compared to placebo (6.7 months vs 4.7 months; P<0.001). This trial indicates that erlotinib is safe and tolerable and can prolong survival in patients after failure of first- or second-line chemotherapy.

Trastuzumab is a humanized monoclonal antibody to *erb*B-2 (*HER*-2/*neu*), currently under investigation for the treatment of lung cancer. The Eastern Cooperative Oncology Group (ECOG) evaluated combination carboplatin, paclitaxel, and trastuzumab in patients with advanced NSCLC[25]. Toxicity with chemotherapy and trastuzumab was no worse than cytotoxic therapy alone. Overall survival is similar to historical data using carboplatin and paclitaxel alone; however, patients with 3+ HER-2/*neu* expression did well in contrast to historical data, suggesting potential benefit for trastuzumab in this subset of patients NSCLC. A randomized phase II trial examined the effect of adding trastuzumab to a standard chemotherapeutic combination (gemcitabine—cisplatin) in patients with *HER-2/neu*-positive NSCLC[26]. In this study, 51 patients were treated with trastuzumab plus gemcitabine—cisplatin and 50 with gemcitabine — cisplatin alone. Efficacy was similar in the trastuzumab and control arms: response rate 36% versus 41%; median progression-free survival (PFS) 6.1 versus 7 months. Response rate (83%) and median PFS (8.5 months) appeared superior in the trastuzumab-treated patients with high expression of HER-2/neu (3+ by fluorescence in *situ* hybridization-positive NSCLC).

The EGFr pathway, including *erb*B-1 and *erb*B-2, represent a promising avenue for treatment of NSCLC, either with antibodies or tyrosine kinase inhibitors. Ongoing studies of these agents, either alone or in combination with cytotoxic chemotherapy, will determine the ultimate role of this strategy.

p53

The human p53 protein is a tumor suppressor nuclear phosphoprotein. p53 activates the growth-arrest pathway to allow DNA repair or the apoptotic pathway leading to programmed cell death[17]. Once p53 genes are deleted or mutated, cells become susceptible to DNA damage and dysregulated cell growth. This is associated with poor prognosis in patients with NSCLC[11] and may also identify patients more likely to be resistant to chemotherapy or radiotherapy[27].

Adenoviral p53 gene therapy has been studied, as a strategy to improve survival with minimal toxicity. The additional benefit from intratumoral adenoviral p53 gene therapy was studied in patients undergoing first-line chemotherapy for advanced NSCLC[28]. In this study, there was no difference between the response rate of lesions treated with p53 gene therapy in addition to chemotherapy (52% objective responses) and lesions treated with chemotherapy alone (48% objective responses). There was no survival difference between the 2 regimens. Intratumoral adenoviral *p53* gene therapy provided no additional benefit in patients receiving an effective first-line chemotherapy for advanced NSCLC[28].

A phase II trial of combination radiotherapy in patients with localized NSCLC revealed an improved pathologic control rate of 62% among eight patients evaluated compared with historical controls receiving chemoradiation or

radiation alone[29]. Five (39%) of 13 patients achieved complete response, whereas two others (15%) had partial responses. However, in a multicenter nonrandomized phase II study of combination chemotherapy and direct intratumor wild-type p53 gene transfer, comparing the isolated responses of treated tumor lesions with a comparable lesion not receiving gene therapy within each patient, there was no additional benefit in patients with advanced NSCLC receiving effective first-line chemotherapy[30]. Like most gene therapy strategies, problems of efficient gene transfer delivery and replication-defective vector spreading remain. In addition, adenoviruses can bind and inactivate wild-type *p53* in normal cells[31].

Cyclooxygenase-2 (COX-2) Enzyme

Cyclooxygenase enzymes function to convert arachidonic acids to prostaglandins. The cyclooxygenase-2 enzyme (COX-2) produces prostaglandin E2 (PGE2), which stimulates bcl-2 and thus inhibits apoptosis. This process results in increased tumor invasion, angiogenesis and metastasis[32]. The frequent expression of *COX-2* in early lesions combined with the known reduction of tumor burden in animals treated with *COX-2* inhibitors before carcinogen exposure indicate that COX-2 could be a promising target for lung cancer chemoprevention[33]. Although single-agent COX-2 inhibitors may have limited utility in the treatment of lung cancer, use of COX-2 inhibitors, is currently being tested in an ongoing pilot phase II chemopreventive trial. In addition, synergistic cytotoxicity has been observed with combination of COX-2 inhibitors and several chemotherapeutic agents, including the taxanes, platinum compounds, and topoisomerase I inhibitors, in NSCLC cell lines[34]. In a phase II clinical trial, 29 patients with stages IB to IIIA NSCLC were treated with two preoperative cycles of paclitaxel and carboplatin, as well as daily celecoxib, followed by surgical resection[35]. There were no complete pathologic responses, but 24% had minimal residual microscopic disease. The addition of celecoxib to paclitaxel and carboplatin may enhance the response to preoperative paclitaxel and carboplatin in patients with NSCLC, compared to historical controls.

Angiogenesis

Tumor-induced neovascularization (angiogenesis) is necessary for both tumor growth and metastatic spread, and a large research effort currently is directed into studying its role in cancer development. Immunohistochemical staining for factor VIII, vascular endothelial growth factor (VEGF), CD-31, and CD-34 can be used to assess microvessels, and number of microvessels in a NSCLC can be used to assess angiogenesis. VEGF, strongly induced by hypoxia, promotes vascular permeability, endothelial cell replication, and migration. By inhibiting angiogenesis, tumor growth and metastatic spread can be controlled.

Both recombinant humanized anti-VEGF antibodies (RhuMAb VEGF) and VEGFr tyrosine kinase inhibitors have been tested in animal models and are being investigated in clinical trials. In animal studies, anti-VEGF antibodies suppressed tumor growth, metastatic spread, and ascites formation in tumor-bearing nude mice but did not cause tumor regression[36].

Hurwitz et al reported that rhuMAb VEGF (bevacizumab) plus chemotherapy resulted in increased survival, progression-free survival, response rate and duration of response, as compared alone in patients with colon cancer, increasing the interest in the study of this agent in patients with other types of cancer, including NSCLC[37]. A randomized study of rhuMAb VEGF was conducted in patients with advanced NSCLC (Stage IIIb with pleural effusion, Stage IV or recurrent disease)[38]. Patients were randomized to carboplatin and paclitaxel (CP) alone, CP plus low-dose rhuMAb VEGF (7.5 mg/kg q3 wks), or CP plus high-dose rhuMAb VEGF (15 mg/kg q3 wks). Sudden and life-threatening hemoptysis occurred in 6 rhuMAb VEGF treated subjects and was fatal in 4; 4/6 occurred in subjects with squamous cell histology. In this study, rhuMAb VEGF (15 mg/kg) in combination with CP chemotherapy was associated with improved response rates and prolonged time to disease progression as compared with carboplatin/paclitaxel chemotherapy alone. Subsequently, a subset analysis of non-squamous (non-SQ) pts was performed[39]. Median survival for the non-SQ population was improved in both rhuMAb VEGF dose groups, and compared favorably with that achieved with CP chemotherapy alone. Thus, treatment of selected patients with NSCLC — non-central, non-squamous — may improve survival with minimal side effects and may represent an important treatment strategy in the future.

Invasion/ Extracellular Matrix Degradation and Cellular Adhesion

Matrix metalloproteinases (MMP) have been implicated in the breakdown of vascular barriers, allowing tumor cells to infiltrate blood vessels. Plasminogen activators are members of the serine protease family. They are responsible for converting plasminogen to plasmin. Plasmin can degrade various proteins in the extracellular matrix. Plasminogen activators are regulated by plasminogen activator inhibitors. Another means through which basement membrane degradation occurs through the plasminogen

activation system is the secretion of urokinase plasminogen activator (uPA) in its inactive form (pro-uPA) by tumor cells. Pro-uPA is converted to its active form (uPA) upon binding to its specific membrane-bound receptor, u-PAR. This activated form of uPA then converts plasminogen into plasmin, which degrades the protein components of the extracellular matrix, such as laminin and fibronectin. Plasmin can also activate pro-enzyme forms of MMPs to further break down the extracellular matrix. U-PAR is expressed on stromal cells as well as tumor cells[17]. Cluster designation 44 (CD-44), an integral membrane glycoprotein, is a receptor for hyaluronan (a component of the extracellular matrix). CD-44 is involved in cell-to-cell and cell-to-extracellular matrix interactions and is correlated with metastatic spread[17].

Several MMP inhibitors (MMPI) against various isoforms have been developed which were reviewed recently[40]. The most-studied of these agents include marimastat, the first orally available synthetic MMPI, and prinomastat. Recent analyses of randomized, placebo-controlled phase III trials of these agents in SCLC and NSCLC revealed no survival benefit or prolongation in time to disease progression[41-43]. The negative results that led to the termination of lung cancer studies with other MMPIs; however, this strategy may represent an effective strategy, in combination with other agents.

SUMMARY

Molecular biologic staging of patients with stage I NSCLC may have the potential to alter therapy, in addition to improving risk stratification. The ability of molecular biologic markers to predict results of chemotherapy would enable the clinician to design therapy based on the individual tumor. In addition, identifying and understanding the mechanisms of treatment resistance offers another pathway to intervene, by blocking or reversing the mechanism of resistance. Furthermore, the understanding of the molecular mechanism of receptor activity and DNA repair enables the study of pharmacologic targeting, with chemotherapy or biologic agents, such as EGFr antibodies or tyrosine kinase inhibitors. Perhaps the most promising area of research is the development of novel drugs whose mechanism of action targets the pathways of various molecular markers.

Molecular biologic staging offers an opportunity to individualize a chemotherapeutic regimen based on the molecular profile of the tumor, thus providing the potential for improved outcomes with less morbidity in patients with both NSCLC. The ultimate power of molecular biologic staging depends on the ability to alter therapy and improve outcome, which has not yet been demonstrated. However, with current technology, it would be possible to biopsy a patient with clinical stage I NSCLC and determine the relative prognosis, based on molecular staging. Patients with strong negative prognostic markers and patients with occult metastases in the bone marrow or serum might be treated with induction biologic therapy or chemotherapy; furthermore, the choice of agents would be determined the biological characteristics of the tumor. This strategy will become even more accurate with the development of "real-time" genetic analysis, such as with reverse transcription polymerase chain reaction (rt-PCR), enabling the analysis of genetic mutations at the time of surgery. In the near future, it is possible that patients with NSCLC will be staged and treated according to a TNMB staging system: Tumor, Nodes, Metastases, and Biology.

REFERENCES

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ. Cancer Statistics 2005. Ca Cancer J Clin 2005;55:10-30.
2. Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111:1710-17.
3. Naruke T, Tsuchiya R, Kondo H, Asamura H. Prognosis and survival after resection for bronchogenic carcinoma based on the 1997 TNM-staging classification: the Japanese experience. Ann Thorac Surg 2001;71: 1759-1764.
4. Non-Small Cell Lung Cancer Clinical Practice Guidelines in Oncology. JNCCN 2004;2:94-124.
5. Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med. 2004;350: 351-360.
6. Strauss GM, Herndon JE, Sherman DD, Mathisen DJ, Carey RW, Choi NC, Rege VB, Modeas C, Green MR. Randomized clinical trial of adjuvant chemotherapy with paclitaxel and carboplatin following resection in stage IB non-small-cell lung cancer (NSCLC): report of cancer and leukemia group B (CALGB) protocol 9633. Proc Am Soc Clin Oncol 2004; abstract 7019.
7. Winton T, Livingston R, Johnson D, Rigas J, Johnston M, Butts C, Cormier Y, Goss G, Inculet R, Vallieres E, Fry W, Bethune D, Ayoub J, Ding K, Seymour L, Graham B, Tsao MS, Gandara D, Kesler K, Demmy T, Shepherd F; National Cancer Institute of Canada Clinical Trials Group; National Cancer Institute of the

United States Intergroup JBR.10 Trial Investigators. A prospective randomized trial of adjuvant vinorelbine (VIN) and cisplatin (CIS) in completely resected stage IB and II non-small-cell lung cancer (NSCLC) intergroup JBR 10. Proc Am Soc Clin Oncol 2004; abstract 7018.

8. D'Cunha J, Corfits AL, Herndon JE 2nd, Kern JA, Kohman LJ, Patterson GA, Kratzke RA, Maddaus MA. Molecular staging of lung cancer: Real-time polymerase chain reaction estimation of lymph node micrometastatic tumor cell burden in stage I non-small cell lung cancer — preliminary results of cancer and leukemia group B trial 9761. J Thorac Cardiovasc Surg 2002;123:484-491.
9. Chen G, Gharib TG, Wang H, Huang CC, Kuick R, Thomas DG, Shedden KA, Misek DE, Taylor JM, Giordano TJ, Kardia SL, Iannettoni MD, Yee J, Hogg PJ, Orringer MB, Hanash SM, Beer DG. Protein profiles associated with survival in lung adenocarcinoma. Proc Nat Acad Sci USA 2003;100:13537-42.
10. Granville CA, Dennis PA. An overview of lung cancer genomics and proteomics. Am J Respir Cell Molec Biol 2005;32:169-176.
11. D'Amico TA, Massey M, Herndon JE 2nd, Moore MB, Harpole DH Jr. A biological risk model for stage I lung cancer: Immunohistochemical analysis of 408 patients with use of ten molecular markers. J Thorac Cardiovasc Surg 1999;117:736-43.
12. D'Amico TA, Aloia TA, Herndon J E, Moore MB, Brooks K, Lau CL and Harpole DH: Molecular biologic substaging in patients with stage I non-small cell lung cancer: Risk stratification according to gender and histologic subtype. Ann Thorac Surg 2000;69: 882-886.
13. Harpole DH Jr, Herndon JE II, Young WG, Wolfe WG, Sabiston DC Jr: Stage I non-small cell lung cancer: A multivariate analysis of treatment methods and patterns. Cancer 1995;76:787-96.
14. Harpole DH Jr, Herndon JE II, Wolfe WG, Iglehart JD, Marks JR. A prognostic model of recurrence and death in stage I non-small cell lung cancer utilizing presentation, histopathology, and oncoprotein expression. Cancer Res 1995;55:51-6.
15. Harpole DH Jr, Richards WG, Herndon JE II, Sugarbaker DJ: Angiogenesis and molecular biologic substaging in patients with stage I non-small cell lung cancer. Ann Thorac Surg 1996;61:1470-6.
16. Brooks KR, To K, Moore-Joshi M, Conlon DH, Herndon JE, D'Amico TA Harpole DH Jr. Measurement of chemotherapy resistance markers in patients with stage III non-small cell lung cancer: A novel approach to patient selection. Ann Thorac Surg 2003; 76:187-93.
17. Lau CL, D'Amico TA, Harpole DH: Staging and prognosis: Clinical and molecular prognostic factors and models for non-small cell lung cancer. In Pass HI, Mitchell JB, Johnson DH, Turrisi AT, Minna JD (eds): Lung Cancer Principles and Practice, (Second Edition), Philadelphia, Lippincott, Williams and Wilkins, 2000, 612-627.
18. Kern JA, Schwartz DA, Nordberg JE. P185neu expression in human lung adenocarcinoma predicts shortened survival. Cancer Res 1990; 50:5184-7.
19. Langer CJ. Emerging role of epidermal growth factor receptor inhibition in therapy for advanced malignancy: focus on NSCLC. Int J Radiat Oncol Biol Phys. 2004; 58:991-1002.
20. Giaccone G, Herbst RS, Manegold C. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 1. J Clin Oncol. 2004;22:777-84.
21. Herbst RS, Giaccone G, Schiller JH. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 2. J Clin Oncol. 2004;22:785-94.
22. Herbst RS, Prager D, Hermann R. TRIBUTE — a phase III trial of erlotinib HCl (OSI-774) combined with carboplatin and paclitaxel (CP) chemotherapy in advanced non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2004; Abstract number 7011.
23. Gatzemeier U, Pluzanska A, Szezesna A. Results of a phase III trial of erlotinib (OSI-744) combined with cisplatin and gemcitabine (GC) chemotherapy in advanced non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2004; Abstract number 7010.
24. Shepherd FA, Pereira J, Ciuleanu TE. A randomized placebo-controlled trial of erlotinib in patients with advanced non-small cell lung cancer (NSCLC) following failure of 1st or 2nd line chemotherapy. A National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) trial. Proc Am Soc Clin Oncol 2004; Abstract number 7022.
25. Langer CJ, Stephenson P, Thor A, Vangel M, Johnson DH. Trastuzumab in the treatment of advanced non-small-cell lung cancer: is there a role? Focus on Eastern Cooperative Oncology Group Study 2598. J Clin Oncol 2004;22:1180-1187.
26. Gatzemeier U, Groth G, Butts C, Van Zandwijk N. Randomized phase II trial of gemcitabine-cisplatin with or without trastuzumab in HER2-positive non-

small-cell lung cancer. Ann Onc 2004;15:19-27.
27. Hamada M, Fujiwara T, Hizuta A, Gochi A, Naomoto Y, Takakura N, Takahashi K, Roth JA, Tanaka N, Orita K. The p53 gene is a potent determinant of chemosensitivity and radiosensitivity in gastric and colorectal cancers. J Cancer Res Clin Oncol 1996;122:360-365.
28. Szak ST. Pietenpol JA. High affinity insertion/deletion lesion binding by p53. Evidence for a role of the p53 central domain. J Biol Chem 1999;274:3904-9.
29. Swisher SG, Roth JA, Komaki R. A phase II trial of adenoviral mediated p53 gene transfer (RPR/INGN 201) in conjunction with radiation therapy in patients with localized non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2000;19:461a, (abstr 1807)
30. Schuler M, Hermann R, De Greve JLP, Stewart AK, Gatzemeier U, Stewart DJ, Laufman L, Gralla R, Kuball J, Buhl R, Heussel CP, Kommoss F, Perruchoud AP, Shepherd FA, Fritz MA, Horowitz JA, Huber C, Rochlitz C. Adenovirus-mediated wild-type p53 gene transfer in patients receiving chemotherapy for advanced non-small-cell lung cancer: Results of a multicenter phase II study. J Clin Oncol 2001;19:1750-1758.
31. Dy GK, Adjei AA. Novel Targets for Lung Cancer Therapy: Part II. J Clin Oncol 2002;20:3016-3028.
32. Fosslien E. Molecular pathology of cyclooxygenase-2 in neoplasia. Ann of Clin Lab Science 2000;30:3-21.
33. Wardlaw SA, March TH, Belinsky SA: Cyclooxygenase-2 expression is abundant in alveolar type II cells in lung cancer-sensitive mouse strains and in premalignant lesions. Carcinogenesis 2000;21:1371-1377.
34. Hida T, Kozaki K, Muramatsu H, Masuda A, Shimizu S, Mitsudomi T, Sugiura T, Ogawa M, Takahashi T. Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. Clin Cancer Res 2000;6:2006-2011.
35. Altorki NK, Keresztes RS, Port JL, Libby DM, Korst RJ, Flieder DB, Ferrara CA, Yankelevitz DF, Subbaramaiah K, Pasmantier MW, Dannenberg AJ. Celecoxib, a selective cyclo-oxygenase-2 inhibitor, enhances the response to preoperative paclitaxel and carboplatin in early-stage non—small-cell lung cancer. J Clin Oncol 2003;21:2645-2650.
36. Schlaeppi JM, Wood JM. Targeting vascular endothelial growth factor (VEGF) for anti-tumor therapy, by anti-VEGF neutralizing monoclonal antibodies or by VEGF receptor tyrosine-kinase inhibitors. Can Met Reviews 1999;18:473-81.
37. Hurwitz H, Fehrenbacher L, Cartwright T, Hainsworth J. Bevacizumab (a monoclonal antibody to vascular endothelial growth factor) prolongs survival in first-line colorectal cancer (CRC): Results of a phase III trial of bevacizumab in combination with bolus IFL (irinotecan, 5-fluorouracil, leucovorin) as first-line therapy in subjects with metastatic CRC. Proc Am Soc Clin Oncol 2003;22a (abstract 3646).
38. DeVore RF, Fehrenbacher L, Herbst RS. A randomized phase II trial comparing rhumAb VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) plus carboplatin/paclitaxel (CP) to CP alone in patients with stage IIIB/IV NSCLC. Proc Am Soc Clin Oncol, 2000;19:485a (abstr 1896).
39. Johnson DH, DeVore R, Kabbinavar F. Carboplatin (C) + paclitaxel (P) + rhuMab-VEGF(AVF) may prolong survival in advanced non-squamous lung cancer. Proc Am Soc Clin Oncol 2001;20:315a (abstr 1256).
40. Hidalgo M, Eckhardt SG. Development of matrix metalloproteinase inhibitors in cancer therapy. J Natl Cancer Inst 2001;93:178-193.
41. Shepherd FA, Giaccone G, Debruyne C. Randomized double-blind placebo-controlled trial of marimastat in patients with small cell lung cancer (SCLC) following response to first-line chemotherapy: An NCIC-CTG and EORTC study. Proc Am Soc Clin Oncol 2001;20: 4a (abstr 11).
42. Smylie M, Mercier R, Aboulafia D. Phase III study of the matrix metalloprotease (MMP) inhibitor prinomastat in patients having advanced non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2001;20:307a (abstr 1226).
43. Leighl NB, Shepherd F Paz-Ares L, Douillard J-Y, Peschel C, Arnold A, Depierre A, Santoro A, Betticher DC, Gatzemeier U, Jassem J, Crawford J, Tu D, Bezjak A, Humphrey JS, Voi M, Galbraith S, Hann K, Seymour L, Shepherd FA. Randomized phase II-III study of matrix metalloproteinase inhibitor (MMPI) BMS-275291 in combination with paclitaxel (P) and carboplatin (C) in advanced non-small cell lung cancer (NSCLC): NCIC-CTG BR.18. J Clin Oncol 2004;22: 7038.