Lung Cancer

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Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researched on the lung cancer.
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1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

Literatures

Ahmed, S. M. and R. Salgia (2006). "Epidermal growth factor receptor mutations and susceptibility to targeted therapy in lung cancer." Respirology **11**(6): 687-92.

According to 2002 estimates, 1.35 million people were diagnosed with and 1.18 million died of lung cancer worldwide. Recently, a new class of medications targeting signal transduction pathways has come into focus in the treatment of various malignancies. In lung cancer, the molecules gefitinib and erlotinib which target the intracellular kinase domain of the epidermal growth factor receptor (EGFR), cause significant tumour responses and, in the case of erlotinib, a survival benefit in patients with previously treated cancers. Responses were most pronounced in female non-smokers with adenocarcinoma histology. These patients were found more likely to harbour mutations of the receptor kinase domain, including in-frame deletions in exon 19 (such as deletions of codons 746-750) and point deletions in exon 21 (such as L858R). Other EGFR kinase domain mutations have been found to confer resistance (T790M) or differential susceptibility to erlotinib and gefitinib (E884K). Gene amplification of EGFR also may predict sensitivity, although the mechanism by which this occurs is unclear, because level of expression detected by immunohistochemistry has not been correlated with increased sensitivity. Phenotypic and genotypic epithelial to mesenchymal transition may be an indicator of resistance to EGFR kinase inhibitors. In this article, we review efforts that have been undertaken to identify genomic determinants of drug susceptibility to EGFR tyrosine kinase inhibitors, with particular focus on the role of gene mutations.

Ahn, M. J., B. B. Park, et al. (2008). "Are there any ethnic differences in molecular predictors of erlotinib efficacy in advanced non-small cell lung cancer?" Clin Cancer Res **14**(12): 3860-6.

PURPOSE: This study investigated possible molecular predictors of outcome in Korean patients with advanced non-small cell lung cancer treated with erlotinib. EXPERIMENTAL DESIGN: One hundred and twenty patients received erlotinib and were followed prospectively. Ninety-two tissue samples were analyzed for epidermal growth factor receptor (EGFR) gene mutations (exons 18, 19, and 21), 88 for EGFR gene amplification by real-time PCR, and 75 for EGFR protein expression by immunohistochemistry. RESULTS: The overall tumor response rate was 24.2% (complete response, 4; partial response, 25) with 56.7% of disease control rate. With a median follow-up of 23.6 months, the median time to progression (TTP) was 2.7 months and the median overall survival was 12.9 months. EGFR gene mutations were found in 26.1% (24 of 92), EGFR gene amplification in 40.9% (36 of 88), and EGFR protein expression in 72% (54 of 75). There was a strong association between EGFR gene mutations and gene amplification (gamma = 0.241). Patients with EGFR gene mutations or gene amplification showed both better response rate (58.3% versus 16.2%, $P < 0.001$; 41.7% versus 17.3%, $P = 0.012$) and TTP (8.6 versus 2.5 months, $P = 0.003$; 5.8 versus 1.8 months, $P < 0.001$) and overall survival (not reached versus 10.8 months, $P = 0.023$; not reached versus 10.1 months, $P = 0.033$). By

multivariate analysis, EGFR gene mutation was the only significant molecular predictor for TTP (hazard ratio, 0.47; 95% confidence interval, 0.25-0.89). CONCLUSIONS: Our findings indicate that EGFR gene mutation is a more predictive marker for improved TTP than EGFR gene amplification in erlotinib-treated Korean non-small cell lung cancer patients. Prospective studies from diverse ethnic backgrounds are required to determine the exact role of these molecular markers.

Al-Kuraya, K., A. K. Siraj, et al. (2006). "High epidermal growth factor receptor amplification rate but low mutation frequency in Middle East lung cancer population." Hum Pathol **37**(4): 453-7.

Epidermal growth factor receptor (EGFR) exon 18-21 mutations were shown to be highly predictive of response to gefitinib (Iressa) therapy in lung cancer. Studies on Western and Japanese lung cancers have indicated substantial differences in the EGFR mutation frequency between these populations. To investigate the prevalence of EGFR in another distinct ethnic group, EGFR alterations were studied in 47 consecutive non small cell lung cancers from Saudi Arabia by immunohistochemistry, fluorescence in situ hybridization, and DNA sequencing. Detectable EGFR expression was seen in 69.8% of 43 interpretable cancers. Epidermal growth factor receptor amplification, present in 15.3% of 39 analyzable cancers, was strongly associated with high levels of EGFR expression (P = .0047). Only 1 exon 18-21 mutation was seen among 34 lung cancers that could be successfully sequenced. It is concluded that EGFR exon 18-21 mutations are rare in Middle East patients with lung cancer and occur in a similar range as in Western patients. The remarkable high rate of EGFR gene amplifications could potentially facilitate studies on the predictive role of gene copy number changes for response to anti-EGFR therapies in Middle East patient sets.

Amann, J., S. Kalyankrishna, et al. (2005). "Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer." Cancer Res **65**(1): 226-35.

Epidermal growth factor receptor (EGFR) is occasionally amplified and/or mutated in non-small cell lung cancer (NSCLC) and can be coexpressed with other members of the HER receptor family to form functional heterodimers. We therefore investigated lung cancer cell lines for alterations in EGFR gene copy number, enhanced expression of EGFR and other HER family members, and EGFR coding sequence mutations and correlated these findings with response to treatment with the EGFR inhibitors and the kinetics of ligand-induced signaling. We show here that somatic deletions in the tyrosine kinase domain of EGFR were associated with increased EGFR gene copy number in NSCLC. Treatment with the specific EGFR tyrosine kinase inhibitors (TKI) gefitinib or erlotinib or the EGFR inhibitory antibody cetuximab induced apoptosis of HCC827, a NSCLC cell line with EGFR gene amplification and an exon 19 deletion. H1819, a NSCLC cell line that expresses high levels of EGFR, ErbB2, and ErbB3 but has wild-type EGFR, showed intermediate sensitivity to TKIs. In both cell lines, ligand-induced receptor tyrosine phosphorylation was delayed and prolonged and AKT was constitutively phosphorylated (but remained inhibitable by EGFR TKI). Thus, in addition to EGFR mutations, other factors in NSCLC cells, such as high expression of ErbB family members, may constitutively activate AKT and sensitize cells to EGFR inhibitors.

An, S. J., Z. H. Chen, et al. (2009). "The -271 G>A polymorphism of kinase insert domain-containing receptor gene regulates its transcription level in patients with non-small cell lung cancer." BMC Cancer **9**: 144.

BACKGROUND: Kinase insert domain-containing receptor (KDR) plays a critical role in the metastasis of cancer and is used as a molecular target in cancer therapy. We investigated the characteristics of the -271 G>A polymorphism of the KDR gene to gain information that may benefit the development of individualized therapies for patients with non-small cell lung cancer (NSCLC). METHODS: The -271 G>A polymorphism of the KDR gene in 106 lung cancer patients and 203 healthy control individuals was analyzed by polymerase chain reaction (PCR) and DNA sequencing methods. Real-time quantitative PCR and immunohistochemical methods were used to evaluate KDR mRNA and protein expression levels, respectively, in frozen tumor specimens. RESULTS: The -271 G>A polymorphism was associated with the mRNA expression level of the KDR gene in tumor tissues (t = 2.178, P = 0.032, independent samples t-test). Compared with the AG/GG genotype, the AA genotype was associated with higher KDR mRNA expression in tumor tissues. We found no relationship between the genotype and the KDR protein expression level and no significant difference in the distribution of the KDR gene polymorphism genotypes between lung cancer patients and the control group (chi2 = 1.269, P = 0.264, Fisher's exact test). CONCLUSION: This study is the first to show that the -271 G>A polymorphism of the KDR gene may be a functional polymorphism related to the regulation of gene transcription. These findings may have important implications for therapies targeting KDR in patients with NSCLC.

Andratschke, N. H., K. H. Dittmann, et al. (2004). "Epidermal growth factor receptor as a target to improve treatment of lung cancer." Clin Lung Cancer **5**(6): 340-52.

Despite considerable efforts to reduce tobacco use, lung cancer remains the most common cancer in both men and women. Recent advances in radiation therapy and chemotherapy for lung cancer have yielded encouraging results, but survival in patients with locally advanced non-small-cell lung cancer (NSCLC) remains poor. As more and more molecular changes and their importance in malignant tissues continue to be characterized, approaches to target those aberrant pathways are being actively explored. The epidermal growth factor receptor (EGFR) is commonly overexpressed in NSCLC, particularly squamous cell carcinoma, and has been implicated in the development and progression of this disease, although a clear correlation with prognosis has not been established. Several different strategies have been developed to target and block the EGFR and its downstream effects, and some of them have been intensively studied in preclinical and clinical studies as a single-agent approach or in combination with radiation therapy or chemotherapy. In this article, we review the role of EGFR in lung cancer, as well as preclinical and clinical data on strategies to interfere with EGFR signaling alone or in combination with chemotherapy, radiation, or both.

Ansari, J., D. H. Palmer, et al. (2009). "Role of tyrosine kinase inhibitors in lung cancer." Anticancer Agents Med Chem **9**(5): 569-75.

Protein tyrosine kinases are enzymes which catalyze the phosphorylation of tyrosine residues and activate a downstream cascade of cellular signalling pathways which regulate cell proliferation, differentiation and apoptosis and a wide variety of cellular functions. Clinical developments over the past decade have identified several novel therapeutic agents which inhibit tyrosine kinase activity, either by direct receptor inhibition or indirect inhibition of tyrosine kinase controlled pathways. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKI), such as gefitinib and erlotinib have been studied extensively in patients with refractory non-small cell lung cancer (NSCLC). Early studies with gefitinib showed undoubted clinical activity but failed to show a survival benefit, whereas studies with erlotinib showed a small but statistically significant benefit in overall survival. Subsequent studies explored the possibility of synergistic activity between targeted agents (gefitinib or erlotinib) and conventional chemotherapy drugs reporting disappointing results. Clinical trial results with gefitinib and erlotinib, either as monotherapy or in combination with chemotherapy, have failed to match the encouraging results noted in the pre-clinical setting. It is now increasingly recognised that clinical exploration of molecular targeted agents may not conform well to traditional phase I/II/III drug trial designs. Therapeutic responses may be limited to a small subpopulation of patients, therefore diluting the overall therapeutic effect. Hypothesising a genetic basis for the heterogeneity in trial results, biomarkers (such as EGFR gene mutation analysis, EGFR protein expression, and increased EGFR gene copy number) have been studied with a view to identifying a target population most likely to benefit from these drugs. Future clinical trials with targeted agents need to be carefully designed to incorporate correlative translational research elements that will allow selection of appropriate treatment strategies for individual patients. For assessment of phase III trial results in advanced disease, progression free survival may serve as a more appropriate end-point than response rate in an adequately designed trial in the appropriately selected population, although there should be no substitute for the overall survival and quality of life end points. The role of EFGR TKI in NSCLC will be discussed in detail and data from these studies will be used to illustrate the challenges in designing clinical trials and interpreting outcomes.

Argiris, A., T. Hensing, et al. (2006). "Combined analysis of molecular and clinical predictors of gefitinib activity in advanced non-small cell lung cancer: epidermal growth factor receptor mutations do not tell the whole story." J Thorac Oncol **1**(1): 52-60.

BACKGROUND: Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors have been introduced in the standard therapy of non-small-cell lung cancer (NSCLC), but they benefit a minority of patients. The study of molecular markers may identify the subset of patients who are the most appropriate to treat with these agents. METHODS: We analyzed 43 patients with advanced NSCLC who were treated with gefitinib, an oral EGFR tyrosine kinase inhibitor, were included in analysis. We evaluated EGFR in tumor tissue by using immunohistochemistry and fluorescence in situ hybridization. We also studied downstream molecules (AKT, ERK, p38 MAPK) and their activation status and the presence of EGFR mutations in tumor tissue in exons 18-21. RESULTS: Three patients had tumors with EGFR mutations, all of which had EGFR gene amplification with a ratio of 2 or greater (p= 0.001). There was no correlation between EGFR protein expression and gene amplification. Six patients (14%) achieved an objective response and nine (21%) had stable disease; the median survival was 162 days. EGFR mutations,

high levels of AKT protein expression, rash of any grade, and no history of smoking were predictive of disease control (objective response plus stable disease). Only 3 of 15 patients (20%) with disease control had an EGFR mutation. On multivariate analysis, rash and AKT were independent predictors of disease control. Patients with rash survived longer than patients without rash. CONCLUSIONS: EGFR mutation-positive tumors are present in a small fraction of patients who achieve disease control with gefitinib. Other molecular markers, such as AKT, need to be further evaluated. Clinical parameters remain major determinants of gefitinib activity in NSCLC.

Asahina, H., K. Yamazaki, et al. (2006). "A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations." Br J Cancer **95**(8): 998-1004.

Retrospective analysis has shown that activating mutations in exons 18-21 of the epidermal growth factor receptor (EGFR) gene are a predictor of response to gefitinib. We conducted a phase II trial to evaluate the efficacy and safety of gefitinib as first-line therapy for advanced non-small cell lung cancer (NSCLC) with EGFR mutations. Patients with stage IIIB or IV chemotherapy-naive NSCLC with EGFR mutation were treated with 250 mg gefitinib daily. For mutational analysis, DNA was extracted from paraffin-embedded tissues and EGFR mutations were analysed by direct sequence of PCR products. Twenty (24%) of the 82 patients analysed had EGFR mutations (deletions in or near E746-A750, n=16; L858R, n=4). Sixteen patients were enrolled and treated with gefitinib. Twelve patients had objective response and response rate was 75% (95% CI, 48-93%). After a median follow-up of 12.7 months (range, 3.1-16.8 months), 10 patients demonstrated disease progression, with median progression-free survival of 8.9 months (95% CI, 6.7-11.1 months). The median overall survival time has not yet been reached. Most of the toxicities were mild. This study showed that gefitinib is very active and well tolerated as first-line therapy for advanced NSCLC with EGFR mutations.

Azuma, K., T. Sasada, et al. (2009). "Expression of ERCC1 and class III beta-tubulin in non-small cell lung cancer patients treated with a combination of cisplatin/docetaxel and concurrent thoracic irradiation." Cancer Chemother Pharmacol **64**(3): 565-73.

INTRODUCTION: The expression of excision repair cross-complementation group 1 (ERCC1) is reported to be correlated with resistance to platinum-based drugs. Class III beta-tubulin is reported to be correlated with resistance to taxanes. METHODS: In the present study, we evaluated whether ERCC1 and class III beta-tubulin expression could be used to predict progression-free and/or overall survival in 34 patients with locally advanced non-small cell lung cancer (NSCLC) receiving concurrent chemoradiation therapy with cisplatin and docetaxel, and immunohistochemistry was used to examine the expression of these two proteins in tumor samples obtained from the patients. RESULTS: Immunostaining for ERCC1 and class III beta-tubulin was positive in 16 and 12 patients, respectively. A significant correlation was observed between ERCC1 expression and response to chemotherapy (P = 0.012), and between class III beta-tubulin expression and histology (P = 0.029). Patients negative for ERCC1 had a significantly longer median progression-free (62.5 vs. 36 weeks, P = 0.009), but not overall (171 vs. 50.5 weeks, P = 0.208), survival than those positive for ERCC1. Expression of class III beta-tubulin was not correlated with progression-free or overall survival (P = 0.563 and P = 0.265, respectively). Multivariate analysis adjusting for possible confounding factors showed that negative ERCC1 expression (hazard ratio = 3.972, P = 0.009) was a significantly favorable factor for progression-free survival. CONCLUSIONS: This retrospective study indicates that immunostaining for ERCC1 may be useful for predicting survival in NSCLC patients receiving concurrent chemoradiotherapy with cisplatin and docetaxel, and can provide information critical for planning personalized chemotherapy.

Azuma, K., T. Sasada, et al. (2009). "Expression of ERCC1 and class III beta-tubulin in non-small cell lung cancer patients treated with carboplatin and paclitaxel." Lung Cancer **64**(3): 326-33.

The combination of carboplatin and paclitaxel is the most commonly used regimen for the treatment of advanced non-small cell lung cancer (NSCLC) patients. The expression of excision repair cross-complementation group 1 (ERCC1) is reported to be correlated with resistance to platinum-based drugs. Class III beta-tubulin is reported to be correlated with resistance to taxanes. We evaluated whether ERCC1 and class III beta-tubulin expression could predict progression-free and/or overall survival in relapsed NSCLC patients treated with carboplatin and paclitaxel. Immunohistochemistry was used to examine the expression of these two proteins in resected lung tumor samples obtained from 45 patients treated with carboplatin and paclitaxel against recurrent tumors after curative resection. Immunostaining for ERCC1 and class III beta-tubulin was positive in 20 and 16 patients, respectively. Patients negative for ERCC1 had a significantly

longer median progression-free (44 weeks vs. 28 weeks, P=0.046) and overall (102 weeks vs. 56 weeks, P=0.010) survival than those positive for ERCC1. Patients negative for class III beta-tubulin expression had a significantly longer median progression-free (40 weeks vs. 35 weeks, P=0.031), but not overall (78 weeks vs. 57 weeks, P=0.087), survival than those positive for class III beta-tubulin expression. In particular, patients negative for both ERCC1 and class III beta-tubulin had significantly longer progression-free (P=0.036) and overall survival (P=0.015), compared with those positive for ERCC1 and/or class III beta-tubulin. In multivariate analysis, negative class III beta-tubulin expression (hazard ratio=1.912, P=0.048) was significantly favorable factor for progression-free survival, and negative ERCC1 expression (hazard ratio=2.580, P=0.014) and better performance status (hazard ratio=3.287, P=0.007) were significantly favorable factors for overall survival. This retrospective study indicates that immunostaining for ERCC1 and class III beta-tubulin may be useful for predicting survival in NSCLC patients receiving carboplatin and paclitaxel against recurrent tumors after curative resection and can provide information critical for planning personalized chemotherapy.

Azzoli, C. G., B. J. Park, et al. (2008). "Molecularly tailored adjuvant chemotherapy for resected non-small cell lung cancer: a time for excitement and equipoise." J Thorac Oncol **3**(1): 84-93.

In patients with previously-untreated, completely-resected pathologic stage II-III non-small cell lung cancer, 4 months of postoperative cisplatin-based chemotherapy reduces the risk of death by approximately 20%. To date, the only prospectively validated prognostic and predictive factor which can be used to guide clinical practice is pathologic stage. Higher stage patients have a worse prognosis, but derive more benefit from adjuvant chemotherapy. Numerous molecular markers are being developed with the potential to help decide which patients to treat with adjuvant chemotherapy, and which drugs to use. This paper will review the molecular markers which are having immediate impact on treatment decisions in routine practice, and which merit further study in the next generation of adjuvant chemotherapy trials.

Bai, H., L. Mao, et al. (2009). "Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer." J Clin Oncol **27**(16): 2653-9.

PURPOSE: Mutations in the epidermal growth factor receptor (EGFR) kinase domain can predict tumor response to tyrosine kinase inhibitors (TKIs) in non-small-cell lung cancer (NSCLC). However, obtaining tumor tissues for mutation analysis is challenging. We hypothesized that plasma-based EGFR mutation analysis is feasible and has value in predicting tumor response in patients with NSCLC. PATIENTS AND METHODS: Plasma DNA samples and matched tumors from 230 patients with stages IIIB to IV NSCLC were analyzed for EGFR mutations in exons 19 and 21 by using denaturing high-performance liquid chromatography. We compared the mutations in the plasma samples and the matched tumors and determined an association between EGFR mutation status and the patients' clinical outcomes prospectively. RESULTS: In 230 patients, we detected 81 EGFR mutations in 79 (34.3%) of the patients' plasma samples. We detected the same mutations in 63 (79.7%) of the matched tumors. Sixteen plasma (7.0%) and fourteen tumor (6.1%) samples showed unique mutations. The mutation frequencies were significantly higher in never-smokers and in patients with adenocarcinomas (P = .012 and P = .009, respectively). In the 102 patients who failed platinum-based treatment and who were treated with gefitinib, 22 (59.5%) of the 37 with EGFR mutations in the plasma samples, whereas only 15 (23.1%) of the 65 without EGFR mutations, achieved an objective response (P = .002). Patients with EGFR mutations had a significantly longer progression-free survival time than those without mutations (P = .044) in plasma. CONCLUSION: EGFR mutations can be reliably detected in plasma DNA of patients with stages IIIB to IV NSCLC and can be used as a biomarker to predict tumor response to TKIs.

Barlesi, F., I. Nanni-Metellus, et al. (2009). "Non-small cell lung cancer-smokers or non-smokers, does it matter?" Lung Cancer **63**(3): 430-2.

Besides epidermal growth factor receptor (EGFR) gene mutations, clinical factors such as smoking status have been identified as predictors for survival for NSCLC patients treated with EGFR-tyrosine kinase inhibitors (TKI). However, the biological screening for EGFR gene mutations is not routinely available everywhere. Therefore, the question arises if the decision to treat patients with EGFR-TKI should be based on clinical factors, and in particular smoking status, alone. We illustrate the difficulties faced by clinicians with the case of a 56-year-old man with stage IV lung adenocarcinoma and a smoking history of 30-pack-year. This patient received erlotinib first-line after its enrolment in a clinical trial. After 4 months, he presented with a dramatic clinical and radiological response. The biological analysis of the tumour revealed an EGFR

exon 19 deletion. This report emphasizes that smoking status alone appears inappropriate in selecting patients for EGFR-TKI treatment. In addition, the relatively high number of (ex-)smokers retrieved from prospective studies on NSCLC patients with tumours showing an EGFR mutation should be emphasized, as it represents up to 30% of patients. Therefore, a biological rather than a clinical selection for patients' eligibility for EGFR-TKI appears mandatory.

Beau-Faller, M., A. M. Ruppert, et al. (2008). "MET gene copy number in non-small cell lung cancer: molecular analysis in a targeted tyrosine kinase inhibitor naive cohort." J Thorac Oncol **3**(4): 331-9.

INTRODUCTION: Recent clinical success of epidermal growth factor (EGFR)-tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer (NSCLC) have raised hopes that targeting other deregulated growth factor signaling, such as the hepatocyte growth factor/MET pathway, will lead to new therapeutic options for NSCLC. Furthermore, NSCLC present secondary EGFR-TKIs resistance related to exons 20 and 19 EGFR mutations or more recently to MET amplification. The aim of this study was to determine MET copy number related to EGFR copy number and K-Ras mutations in a targeted TKI naive NSCLC cohort. METHODS: We investigated 106 frozen tumors from surgically resected NSCLC patients. Genes copy number of MET and EGFR were assessed by quantitative relative real-time polymerase chain reaction and K-Ras mutations by sequencing. RESULTS: MET is amplified in 22 cases (21%) and deleted in nine cases (8.5%). EGFR is amplified in 31 cases (29%). K-Ras is mutated in 11 cases (10.5%). As observed for EGFR amplification, MET amplification is never associated with K-Ras mutation. MET amplification could be associated with EGFR amplification. MET amplification is not related to clinical and pathologic features. MET amplification and EGFR amplification showed a trend toward poor prognosis in adenocarcinomas. CONCLUSION: In EGFR-TKIs naive NSCLC patients, MET amplification is a frequent event, which could be associated with EGFR amplification, but not with K-Ras mutation. MET amplification may identify a subset of NSCLC for new targeted therapy. It will also be important to evaluate MET copy number to properly interpret future clinical trials.

Bell, D. W., T. J. Lynch, et al. (2005). "Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials." J Clin Oncol **23**(31): 8081-92.

PURPOSE: Most cases of non-small-cell lung cancer (NSCLC) with dramatic responses to gefitinib have specific activating mutations in the epidermal growth factor receptor (EGFR), but the predictive value of these mutations has not been defined in large clinical trials. The goal of this study was to determine the contribution of molecular alterations in EGFR to response and survival within the phase II (IDEAL) and phase III (INTACT) trials of gefitinib. PATIENTS AND METHODS: We analyzed the frequency of EGFR mutations in lung cancer specimens from both the IDEAL and INTACT trials and compared it with EGFR gene amplification, another genetic abnormality in NSCLC. RESULTS: EGFR mutations correlated with previously identified clinical features of gefitinib response, including adenocarcinoma histology, absence of smoking history, female sex, and Asian ethnicity. No such association was seen in patients whose tumors had EGFR amplification, suggesting that these molecular markers identify different biologic subsets of NSCLC. In the IDEAL trials, responses to gefitinib were seen in six of 13 tumors (46%) with an EGFR mutation, two of seven tumors (29%) with amplification, and five of 56 tumors (9%) with neither mutation nor amplification (P = .001 for either EGFR mutation or amplification v neither abnormality). Analysis of the INTACT trials did not show a statistically significant difference in response to gefitinib plus chemotherapy according to EGFR genotype. CONCLUSION: EGFR mutations and, to a lesser extent, amplification appear to identify distinct subsets of NSCLC with an increased response to gefitinib. The combination of gefitinib with chemotherapy does not improve survival in patients with these molecular markers.

Bepler, G., M. Begum, et al. (2008). "Molecular analysis-based treatment strategies for non-small cell lung cancer." Cancer Control **15**(2): 130-9.

BACKGROUND: Lung cancer is the leading cause of cancer-related mortality. Improved understanding in the molecular biology and genetics of lung cancer has resulted in the identification of individual genes, gene expression profiles, and molecular pathways that may be useful for clinical management decisions. METHODS: We focused on recent molecules and platforms under evaluation for implementation into clinical decision making. RESULTS: Prognostic molecular parameters are defined as markers that impact overall outcome in terms of survival independent of therapeutic interventions. Predictive molecular parameters are defined as markers that impact therapeutic efficacy. CONCLUSIONS: Several molecules and profiles are emerging with promising utility as predictive and prognostic parameters in non-small cell lung cancer independent of the standard clinical parameters, such as stage, performance status, and gender. These

include the genes ERCC1, RRM1, and BRCA1, which are involved in nucleotide metabolism and DNA damage repair, epidermal growth factor receptor, which is involved in cell proliferation and survival, and oligonucleotide expression array profiles, which are signatures of global gene expression associated with specific tumor phenotypes.

Bieniasz, M., K. Oszajca, et al. (2009). "The positive correlation between gene expression of the two angiogenic factors: VEGF and BMP-2 in lung cancer patients." Lung Cancer **66**(3): 319-26.

Lung cancer is a particular challenge in oncology. More than 1 million new cases occur worldwide every year and despite many clinical trials and modern diagnostic techniques, long-term survival rate has only marginally improved. The aim of the current research is to explore new molecular prognostic factors and identify new targets for anticancer therapy. Current evidence shows that angiogenesis is controlled by several angiogenic factors including VEGF and BMP-2. It has been also demonstrated that VEGF plays a key role in this process that is essential in carcinogenesis. Our study has shown that the expressions of the VEGF, BMP-2 and BMP-4 mRNAs were significantly higher (7.1-fold, 25.6-fold and 2.3-fold, respectively) in lung cancer samples than in adjacent normal lung tissues (real-time RT-PCR). Analysis based on the Pearson's correlation coefficient indicated the positive correlation between VEGF and BMP-2 gene expression, whereas no significant correlation between VEGF and BMP-4 gene expression was found. The mean+/-standard deviation serum level of VEGF was 423+/-136 pg/ml. Significant differences in the serum levels of VEGF between patients with T1 tumors and patients with T2, T3 or T4 tumors were observed. Patients with T2, T3 and T4 tumors, respectively, had 1.6-fold, 1.8-fold and 2.3-fold greater serum levels of VEGF than their peers with T1 tumors. In current study patients homozygous for the 936T allele of the +936C/T VEGF gene polymorphism had 12-fold lower VEGF gene expression and 1.3-fold lower VEGF serum level than patients homozygous for the 936C allele. In conclusion, our findings underline the importance of the two angiogenic factors namely VEGF and BMP-2 as well as +936C/T VEGF gene polymorphism in the evaluation of lung cancer patients.

Brabender, J., H. Usadel, et al. (2003). "Quantitative O(6)-methylguanine DNA methyltransferase methylation analysis in curatively resected non-small cell lung cancer: associations with clinical outcome." Clin Cancer Res **9**(1): 223-7.

PURPOSE: Hypermethylation of the O(6)-methylguanine DNA methyltransferase (MGMT) promoter region leads to transcriptional repression of the MGMT gene and is a common event in primary human neoplasia. The purpose of this study was to determine the frequency and clinical relevance of MGMT gene promoter hypermethylation in curatively resected non-small cell lung cancer (NSCLC). EXPERIMENTAL DESIGN: MGMT hypermethylation, expressed as the ratio between methylated MGMT to unmethylated MYOD1 in genomic DNA, was analyzed in normal and matching tumor tissue from 90 patients with NSCLC, and a control group of 10 patients without cancer using a methylation-specific fluorogenic Real-Time PCR (Taqman) system. RESULTS: Hypermethylation of the MGMT promoter was detected in 34 of 90 (38%) tumor specimens and 16 of 90 (18%) matching normal lung tissues of patients with NSCLC, and in 0 (0%) cases of the control group without lung cancer. The mean MGMT methylation level was significantly higher in tumor than in matching normal tissue ($P < 0.001$). MGMT methylation in normal tissue was always accompanied with MGMT methylation in matching tumor tissue. Patients without MGMT promoter hypermethylation showed a significantly better survival than patients with MGMT promoter hypermethylation ($P = 0.017$). Multivariate analysis revealed MGMT promoter methylation as an independent unfavorable prognostic factor ($P = 0.030$). CONCLUSIONS: MGMT promoter hypermethylation is a common event in patients with primary NSCLC. This epigenetic alteration is associated with inferior survival, suggesting that MGMT promoter hypermethylation might be an important biomarker for a biological aggressive disease in NSCLC.

Brambilla, E. and A. Gazdar (2009). "Pathogenesis of lung cancer signalling pathways: roadmap for therapies." Eur Respir J **33**(6): 1485-97.

Lung cancer is the major cancer killer worldwide, and 5-yr survival is extremely poor (<or=15%), accentuating the need for more effective therapeutic strategies. Significant advances in lung cancer biology may lead to customised therapy based on targeting specific genes and pathways. The main signalling pathways that could provide roadmaps for therapy include the following: growth promoting pathways (Epidermal Growth Factor Receptor/Ras/PhosphatidylInositol 3-Kinase), growth inhibitory pathways (p53/Rb/P14(ARF), STK11), apoptotic pathways (Bcl-2/Bax/Fas/FasL), DNA repair and immortalisation genes. Epigenetic changes in lung cancer contribute strongly to cell transformation by modifying chromatin structures and

the specific expression of genes; these include DNA methylation, histone and chromatin protein modification, and micro-RNA, all of which are responsible for the silencing of tumour suppressor genes while enhancing expression of oncogenes. The genetic and epigenetic pathways involved in lung tumorigenesis differ between smokers and nonsmokers, and are tools for cancer diagnosis, prognosis, clinical follow-up and targeted therapies.

Brueckl, W. M., A. Schoeberl, et al. (2008). "Increased vascular-endothelial growth factor (VEGF) tumor expression and response to epidermal growth factor receptor (EGF-R) inhibitor erlotinib in non-small cell lung cancer (NSCLC)." J Thorac Oncol **3**(3): 314-6.

A 37-year-old female never smoker with metastatic large cell carcinoma of the lung had a partial response to a second line palliative therapy with the EGF-R tyrosine kinase inhibitor erlotinib after platinum based first line therapy failed. Molecular analysis of the primary and a liver metastasis did neither find any EGF-R mutation nor an EGF-R amplification. However, both the primary and the metastasis showed an increased gene expression of vascular-endothelial growth factor-A in contrast to normal tissue, which was confirmed by immunohistochemistry. To our knowledge, this is the first report about a high vascular-endothelial growth factor-A expression in the tumor of a patient responding to an EGF-R inhibitor postulating that there might be a link between both tyrosine kinase pathways.

Buckingham, L. E., J. S. Coon, et al. (2007). "The prognostic value of chromosome 7 polysomy in non-small cell lung cancer patients treated with gefitinib." J Thorac Oncol **2**(5): 414-22.

INTRODUCTION: Specific subpopulations of non-small cell lung cancer (NSCLC) patients defined by clinical features and molecular profiles seem to derive greater benefit from epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, but no general consensus on molecular testing to optimize treatment has emerged. The objective of this study was to evaluate chromosome 7 polysomy and other potential indicators of gefitinib efficacy in advanced NSCLC patients. METHODS: Paraffin-embedded tumors from 82 patients treated with gefitinib were analyzed by immunohistochemistry for expression of EGFR and other markers, and by fluorescence in situ hybridization for EGFR gene or chromosome copy number. Mutational status was assessed by single-strand conformational polymorphism, sequence-specific polymerase chain reaction, and direct sequencing. Molecular and clinical characteristics were evaluated in relation to objective response (OR), progression-free survival (PFS), and overall survival (OS). RESULTS: EGFR mutational status ($p = 0.002$), never smoking ($p = 0.052$), and chromosome 7 polysomy ($p = 0.029$) were significant indicators of OR. EGFR mutation, pAKT or PTEN expression, and chromosome 7 polysomy were associated with longer OS. There was a significant difference in OS between the chromosome 7 polysomy groups ($p = 0.015$) and the groups with both chromosome 7 polysomy and pAkt ($p = 0.002$) and both chromosome7 polysomy and PTEN ($p = 0.04$). In a stepwise proportional hazards analysis, chromosome 7 polysomy and PTEN expression were both significantly associated with longer OS ($p = 0.004$ and 0.017 respectively). CONCLUSION: These results suggest that further study of chromosome 7 polysomy and of pAKT and PTEN expression in patients treated with EGFR tyrosine kinase inhibitors is warranted in developing a clinical test for selecting patients for gefitinib therapy.

Camps, C., R. Sirera, et al. (2006). "Quantification in the serum of the catalytic fraction of reverse telomerase: a useful prognostic factor in advanced non-small cell lung cancer." Anticancer Res **26**(6C): 4905-9.

The purpose of this analysis was to study the association between the quantity of free circulating DNA and clinical variables in 99 advanced non-small cell lung cancer patients (NSCLC). The quantification in the serum of the gene of the catalytic fraction of telomerase (hTERT) by RT-PCR was used as a reference of the total amount of free DNA in blood. Patients were treated with cisplatin and docetaxel. The median hTERT level for patients in stage IIIB was 70.7 ng/ml vs. 53.1 ng/ml in patients in stage IV ($p= 0.35$). There was no association between hTERT values and therapy response, 53.9 ng/ml in the complete response (CR) + partial response (PR) group vs. 54.1 ng/ml in the stable disease (SD) + progressive disease (PD) group ($p=0.23$). In the multivariate analysis, hTERT was an independent predictive variable for time to progression (TTP) Hazard ratio (HR) 2.0, CI 95% 1.2-3.4, $p=0.009$) and overall survival (OS) (HR 2.4 CI 95% 1.3-4.3, $p=0.004$). The analysis of TTP and OS with a cut-off of hTERT at 40 ng/ml revealed that patients about this level had statistically poorer TTP (4 vs. 7 months, $p= 0.009$) and OS (5 vs. 15 months, $p<0.0001$). In conclusion, in advanced NSCLC, high serum hTERT levels may be a poor prognostic indicator for TTP and OS.

Cappuzzo, F., F. R. Hirsch, et al. (2005). "Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer." J Natl Cancer Inst **97**(9): 643-55.

BACKGROUND: Gefitinib is a selective inhibitor of the epidermal growth factor (EGFR) tyrosine kinase, which is overexpressed in many cancers, including non-small-cell lung cancer (NSCLC). We carried out a clinical study to compare the relationship between EGFR gene copy number, EGFR protein expression, EGFR mutations, and Akt activation status as predictive markers for gefitinib therapy in advanced NSCLC. METHODS: Tumors from 102 NSCLC patients treated daily with 250 mg of gefitinib were evaluated for EGFR status by fluorescence in situ hybridization (FISH), DNA sequencing, and immunohistochemistry and for Akt activation status (phospho-Akt [P-Akt]) by immunohistochemistry. Time to progression, overall survival, and 95% confidence intervals (CIs) were calculated and evaluated by the Kaplan-Meier method; groups were compared using the log-rank test. Risk factors associated with survival were evaluated using Cox proportional hazards regression modeling and multivariable analysis. All statistical tests were two-sided. RESULTS: Amplification or high polysomy of the EGFR gene (seen in 33 of 102 patients) and high protein expression (seen in 58 of 98 patients) were statistically significantly associated with better response (36% versus 3%, mean difference = 34%, 95% CI = 16.6 to 50.3; P<.001), disease control rate (67% versus 26%, mean difference = 40.6%, 95% CI = 21.5 to 59.7; P<.001), time to progression (9.0 versus 2.5 months, mean difference = 6.5 months, 95% CI = 2.8 to 10.3; P<.001), and survival (18.7 versus 7.0 months, mean difference = 11.7 months, 95% CI = 2.1 to 21.4; P = .03). EGFR mutations (seen in 15 of 89 patients) were also statistically significantly related to response and time to progression, but the association with survival was not statistically significant, and 40% of the patients with mutation had progressive disease. In multivariable analysis, only high EGFR gene copy number remained statistically significantly associated with better survival (hazard ratio = 0.44, 95% CI = 0.23 to 0.82). Independent of EGFR assessment method, EGFR+/P-Akt+ patients had a statistically significantly better outcome than EGFR-, P-Akt-, or EGFR+/P-Akt- patients. CONCLUSIONS: High EGFR gene copy number identified by FISH may be an effective molecular predictor for gefitinib efficacy in advanced NSCLC.

Cappuzzo, F., C. Ligorio, et al. (2007). "EGFR and HER2 gene copy number and response to first-line chemotherapy in patients with advanced non-small cell lung cancer (NSCLC)." J Thorac Oncol **2**(5): 423-9.

BACKGROUND: A critical point in designing clinical trials comparing chemotherapy with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) in patients with non-small cell lung cancer (NSCLC) is the expected benefit with standard chemotherapy in presence of biological features indicative of TKI sensitivity. The aim of this study was to assess whether EGFR and HER2 gene copy number and Akt activation are associated with response to first-line chemotherapy. METHODS: Tumor samples from 190 patients with NSCLC were analyzed. EGFR and HER2 gene copy number were evaluated by fluorescence in situ hybridization in 185 and 184 cases, respectively. Akt activation was assessed by immunohistochemistry (n = 176). Additional biomarkers included EGFR DNA sequencing (n = 65), and EGFR immunohistochemistry (n = 185). RESULTS: Response rate was not associated with EGFR, HER2, and P-Akt status, irrespective of the method used for biomarker assessment. Among patients with EGFR gene mutations, response to chemotherapy was observed only in individuals with exon 19 deletion (response rate: 46.6% versus 0%, p = 0.02). Among the 190 patients analyzed, 123 received a treatment with a TKI as second- or third-line therapy. When assessed by fluorescence in situ hybridization or DNA sequencing, EGFR-positive patients seemed to be more sensitive to TKIs than to chemotherapy in terms of response rate and time to progression, whereas in EGFR-negative patients, response rate and time to progression favored chemotherapy. CONCLUSION: This study suggested that EGFR expression and gene copy number, HER2 gene copy number, and P-Akt expression are not associated with response to first-line chemotherapy in NSCLC. Prospective phase III trials should compare standard chemotherapy with a TKI in selected NSCLC.

Cappuzzo, F., A. Marchetti, et al. (2009). "Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients." J Clin Oncol **27**(10): 1667-74.

PURPOSE: To investigate the prognostic role of genomic gain for MET and epidermal growth factor receptor (EGFR) genes in surgically resected non-small-cell lung cancer (NSCLC). PATIENTS AND METHODS: This retrospective study included 447 NSCLC patients with available tumor tissue from primary lung tumor and survival data. EGFR and MET status was evaluated by fluorescent in situ hybridization (FISH) in tissue microarray sections. RESULTS: EGFR FISH results were obtained in 376 cases. EGFR gene amplification and high polysomy (EGFR FISH+) were observed in 10.4% and 32.4% of cases, respectively. EGFR FISH-positive patients had a nonsignificant shorter survival than EGFR FISH-negative patients (P = .4). Activating EGFR mutations

were detected in 9.7% of 144 stage I-II disease with no impact on survival. MET FISH analysis was performed in 435 cases. High MET gene copy number (mean > or = 5 copies/cell) was observed in 48 cases (MET+, 11.1%), including 18 cases with true gene amplification (4.1%). MET+ status was associated with advanced stage (P = .01), with grade 3 (P = .016) and with EGFR FISH+ result (P < .0001). No patient with activating EGFR mutation resulted MET+. In the whole population, MET-positive patients had shorter survival than MET-negative patients (P = .005). Multivariable model confirmed that MET-negative patients had a significant reduction in the risk of death than MET-positive patients (hazard ratio, 0.66; P = .04). CONCLUSION: MET increased gene copy number is an independent negative prognostic factor in surgically resected NSCLC. EGFR gene gain does not impact survival after resection.

Cappuzzo, F., L. Toschi, et al. (2005). "HER3 genomic gain and sensitivity to gefitinib in advanced non-small-cell lung cancer patients." Br J Cancer **93**(12): 1334-40.

In non-small-cell lung cancer (NSCLC), sensitivity to tyrosine kinase inhibitors (TKIs) is associated with activating mutations and genomic gain of the epidermal growth factor receptor (EGFR). Preclinical data suggested that HER3 overexpression increases sensitivity to TKIs. A total of 82 NSCLC patients treated with gefitinib (250 mg), and previously evaluated for EGFR and HER2 status by fluorescence in situ hybridisation (FISH) and DNA sequencing, and for Phospho-Akt status by immunohistochemistry, were investigated for HER3 genomic gain by FISH. Patients with high polysomy and gene amplification were considered as HER3 FISH positive (+). HER3 FISH+ pattern was significantly associated with female gender (P=0.02) and never smoking history (P=0.02). Patients with HER3+ tumours (26.8%) had a significantly longer time to progression (3.7 vs 2.7, P=0.04) than patients with HER3- tumours, but not a significantly better response rate or survival. Patients with EGFR+/HER3+ tumours had higher objective response rate (36.4 vs 9.9%, P=0.03) and time to progression (7.7 vs 2.7 months, P=0.03) than patients with EGFR- and/or HER3- tumours, but no significantly longer survival. No difference in response was observed according to HER3 status in patients with EGFR+ tumours. Patients with HER2+/HER3+ tumours had similar outcome as patients with HER2- and/or HER3- tumours. Significantly different clinical end points were not observed between patients with HER3+/P-Akt+ and HER3- and/or P-Akt- tumours. Genomic gain for HER3 is not a marker for response or resistance to TKI therapy in advanced NSCLC patients.

Cappuzzo, F., M. Varella-Garcia, et al. (2009). "MYC and EIF3H Coamplification significantly improve response and survival of non-small cell lung cancer patients (NSCLC) treated with gefitinib." J Thorac Oncol **4**(4): 472-8.

BACKGROUND: We investigated the incidence of eukaryotic translation initiation factor 3 subunit H (EIF3H) and MYC amplification in non-small cell lung cancer (NSCLC) patients, and whether MYC/EIF3H increased gene copy number affected response to Epidermal Growth Factor Receptor tyrosine kinase inhibitors. METHODS: Metastatic NSCLC patients (n = 54) treated with gefitinib were analyzed for the genomic content of EIF3H and MYC genes by fluorescence in situ hybridization (FISH) using a custom-designed 3-color DNA probe set. RESULT: Amplification of EIF3H (ratio EIF3H/CEP8 >2), was observed in 10 cases (18.5%), and MYC was coamplified in all. MYC amplification without coamplification of EIF3H was observed in 2 cases (3.7%). Receiver operating characteristic analysis was conducted to identify the cutoff for MYC and EIF3H copy number best discriminating sensitive and resistant populations. MYC FISH positive patients (MYC+, mean > or =2.8) had a significantly higher response rate (p = 0.003), longer time to progression (p = 0.01) and overall survival (OS: p = 0.02) than MYC- (mean <2.8). Similarly, EIF3H FISH positive patients (EIF3H+, mean > or =2.75) had a significantly higher response rate (p = 0.002), longer time to progression (p = 0.01) and OS (p = 0.01) than EIF3H- (mean <2.75). CONCLUSION: Our results indicate that MYC and EIF3H are frequently coamplified in NSCLC and that a high copy number correlates with increased epidermal growth factor receptor tyrosine kinase inhibitors sensitivity.

Cappuzzo, F., M. Varella-Garcia, et al. (2005). "Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients." J Clin Oncol **23**(22): 5007-18.

PURPOSE: In non-small-cell lung cancer (NSCLC), response to tyrosine kinase inhibitors (TKIs) is significantly associated with the presence of increased copy number and/or activating mutations of the epidermal growth factor receptor gene (EGFR). Preclinical data indicate that HER2, a member of the EGFR family, could enhance TKI sensitivity. PATIENTS AND METHODS: HER2 gene copy numbers per cell were evaluated by fluorescent in situ hybridization (FISH) in 102 NSCLC patients treated with gefitinib, and previously evaluated for EGFR

status by FISH, immunohistochemistry, and presence of mutations. RESULTS: Patients with HER2 high copy number (high polysomy and gene amplification [HER2 FISH positive]) represented 22.8% of patients, and compared with patients with no or low gain (HER2 FISH negative), had significantly better objective response (OR, 34.8% v 6.4%; P = .001), disease control rate (DCR, 56.5% v 33.3%; P = .04), time to progression (TTP, 9.05 v 2.7 months; P = .02), and a trend toward longer overall survival (OS, 20.8 v 8.4 months; P = .056). HER2 protein expression investigated by immunohistochemistry was positive in only five of 72 (7%) patients analyzed and all 89 patients tested by DNA sequencing were negative for mutations in HER2 exon 20. Patients with HER2 FISH-positive tumors displaying increased expression of EGFR protein, gene gain, or mutations (EGFR positive) had a significantly better OR, DCR, TTP, and OS than patients negative for both receptors. CONCLUSION: Increased copy number of the HER2 gene is associated with gefitinib sensitivity in EGFR-positive patients, supporting use of HER2 FISH analysis for selection of patients for TKI therapy.

Ceppi, P., M. Longo, et al. (2008). "Excision repair cross complementing-1 and topoisomerase IIalpha gene expression in small-cell lung cancer patients treated with platinum and etoposide: a retrospective study." J Thorac Oncol **3**(6): 583-9.

HYPOTHESIS: Aim of the study was to quantify ERCC1, RRM1, and TopoIIalpha mRNA expression profile as predictive factors for response and survival in SCLC patients treated with platinum/etoposide. METHODS: Total RNA was extracted from microdissected sections of 103 formalin-fixed, paraffin embedded biopsies. Relative quantification was performed by real-time polymerase chain reaction (PCR) using intron-spanning probes. RESULTS: Eighty-five samples (83%) were successfully amplified. Median overall survival (OS) was 9.9 months; 45 patients had limited disease (LD) (OS = 13.1) and 40 had extensive disease (ED) (OS = 7.1). Fifty-six (65%) patients had an objective response to treatment. A gene expression was detectable in all samples and a correlation between ERCC1 and RRM1 (Rs = 0.34, p = 0.0011) was found. According to response to treatment, it was found that lower TopoIIalpha expression was associated to a better response in LD patients (p = 0.025) and, more interestingly, those who had a complete response had lower TopoIIalpha than both partial and nonresponsive patients (p = 0.015). At univariate analysis LD patients with low ERCC1 had significantly longer survival (median survival 14.9 versus 9.9, p = 0.012), whereas RRM1 and TopoIIalpha levels showed no influence on outcome. At the multivariate analysis, ERCC1 was confirmed to be an independent prognostic factor for survival in LD patients. No significant role was found for ERCC1, RRM1 and TopoIIalpha in ED patients. CONCLUSIONS: ERCC1 and TopoIIalpha are candidate markers in predicting clinical outcome and response to treatment in LD SCLC patients and are worth of further investigation in a prospective study.

Ceppi, P., M. Volante, et al. (2006). "ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine." Ann Oncol **17**(12): 1818-25.

BACKGROUND: Pivotal studies indicate a role of excision repair cross-complementation 1 (ERCC1) gene and ribonucleotide reductase M1 (RRM1) gene in conferring a differential sensitivity to cytotoxic chemotherapy and epidermal growth factor receptor (EGFR) gene has been recently extensively investigated in non-small-cell lung cancer (NSCLC). DESIGN: Formalin-fixed, paraffin-embedded bronchoscopic/fine needle aspiration biopsies obtained from 70 patients with advanced NSCLC were retrospectively collected to investigate the expression level of ERCC1, RRM1 and EGFR by real-time PCR. Sufficient amounts of messenger RNA (mRNA) were successfully extracted from 61 (87%) specimens, reverse transcribed and amplified with intron-spanning primers. Forty-one patients had stage IV disease and 43 received cisplatin/gemcitabine chemotherapy. RESULTS: A strong correlation between ERCC1 and RRM1 mRNA levels (r(s) = 0.624, P < 0.0001) was found. Median survival time in patients with low ERCC1 was significantly longer (17.3 versus 10.9, P = 0.0032 log-rank test) as well as in patients with low RRM1 (13.9 versus 10.9, P = 0.0390 log-rank test). Concomitant low expression levels of ERCC1 and RRM1 (n = 33) were predictive of a better outcome (14.9 versus 10.0, P = 0.0345 log-rank test). Among cisplatin-treated patients, a low ERCC1 level was highly predictive of a longer survival (23.0 versus 12.4, P = 0.0001 log-rank test). No correlation between gene expression levels and histology was reported. No significant correlation between EGFR expression level and survival was found. At multivariate analysis, performance status, response to chemotherapy, presence of weight loss and ERCC1 were independent prognostic factors for survival. CONCLUSIONS: This retrospective study further validates ERCC1 and RRM1 genes as reliable candidates for customized chemotherapy and shows a higher impact on the survival of NSCLC patients treated with cisplatin/gemcitabine for ERCC1. Prospective pharmacogenomic studies represent a research priority in early and advanced NSCLC.

Chang, Y. S., L. Wang, et al. (2004). "Mechanisms underlying lack of insulin-like growth factor-binding protein-3 expression in non-small-cell lung cancer." Oncogene **23**(39): 6569-80.

Expression of insulin-like growth factor-binding protein-3, which (IGFBP-3) inhibits the proliferation of non-small-cell lung cancer (NSCLC) cells by inducing apoptosis, is lost in about half of stage I NSCLC cases. Since promoter methylation can silence gene expression, we investigated whether hypermethylation of the IGFBP-3 promoter is involved in loss of IGFBP-3 expression in NSCLC. We found the IGFBP-3 promoter to be methylated in seven of 13 NSCLC cell lines and in 16 of 23, seven of 9, eight of 11, and six of six tumor specimens from patients with stage I, II, III, and IV NSCLC, respectively. Methylation status correlated with IGFBP-3 mRNA and protein levels in a subset of NSCLC cell lines tested in our study. However, treatment with 5'-aza-2'-deoxycytidine (5'-aza-dC) restored IGFBP-3 expression in four of seven NSCLC cell lines with the methylated promoter, suggesting that multiple mechanisms regulate IGFBP-3 expression in NSCLC. Gel shift and chromatin immunoprecipitation assays showed that methylation of the Sp-1/Sp-3-binding element in the IGFBP-3 promoter influenced the binding of Sp-1, methyl-CpG-binding protein-2 (MeCP2), and histone deacetylase (HDAC). A luciferase construct expressing IGFBP-3 promoter in which the Sp-1/Sp-3 binding element was methylated showed significantly reduced transcriptional activity. The reduction in promoter activity was further suppressed by overexpression of MeCP2, which was rescued by 5'-aza-dC. Thus interference with Sp-1 transactivation by MeCP2 may contribute to the transcriptional defect of IGFBP-3 expression in NSCLC cells with methylated promoter.

Che, G., J. Chen, et al. (2006). "Transfection of nm23-H1 increased expression of beta-Catenin, E-Cadherin and TIMP-1 and decreased the expression of MMP-2, CD44v6 and VEGF and inhibited the metastatic potential of human non-small cell lung cancer cell line L9981." Neoplasma **53**(6): 530-7.

Nm23 is a metastasis suppressor gene. In this report, we transfected nm23-H1 cDNA into L9981, a human large cell lung cancer cell line with nm23 negative expression, and made a stable transfectant. L9981-nm23-H1 cells exhibited lower cells proliferation rate, more G0/G1 phase growth and an increase in apoptosis with a dramatic decreased in the tumor cells ability to metastasize. L9981-nm23-H1 cells also demonstrated a significantly reduced lymph node and pulmonary metastatic capacity in vivo when injected into the nude mice. Furthermore, we used DNA microarray analysis to explore the change in expression of the metastasis-related genes in L9981-nm23-H1 cells. We found that the expression of beta-Catenin, E-Cadherin and TIMP-1 were significantly increased while expression MMP-2, CD44v6, and VEGF was dramatically decreased in L9981-nm23-H1, as confirmed by RT-PCR and western blot. These results demonstrated that nm23-H1 can suppress the mobility and metastatic capacity of cancer cells and the molecular mechanism by which nm23-H1 suppresses tumor metastasis may be via increasing the expression of metastasis-related genes such as beta-Catenin, E-Cadherin and TIMP-1 and decreasing the expression of MMP-2, CD44V6 and VEGF.

Chen, M. F., W. C. Chen, et al. (2006). "p53 status is a major determinant of effects of decreasing peroxiredoxin I expression on tumor growth and response of lung cancer cells to treatment." Int J Radiat Oncol Biol Phys **66**(5): 1461-72.

PURPOSE: The potential roles of peroxiredoxin (Prx) I in carcinogenesis and treatment have been explored. Our previous study revealed differences between A549 (functional p53) and H1299 (null p53) Prx I antisense transfectants. The discrepancy might have resulted from the p53 status. In this study, we further investigated the role of Prx I and p53 on lung cancer growth and the response to treatment in vitro and in vivo. METHODS: We established stable A549 and H1299 transfectants with Prx I antisense and p53, respectively. We then examined their characteristics in vitro and used nude mice xenografts of these cell lines to compare their capacity for tumor invasion and spontaneous metastasis and their sensitivity to radiotherapy. RESULTS: Increased reactive oxygen species caused by lower Prx I activity induced p53 expression. In lethal stress, the augmentation of reactive oxygen species was partially reversed by blocking p53 in A549 with Prx I antisense. We demonstrated the potential contribution of p53-dependent mechanisms to inhibit lung tumor growth and increase radiosensitization using H1299 transfected with p53 in vitro and in vivo. An increased p53 level attenuated the capacity of the cells for metastasis by decreasing vascular endothelial growth factor and induced radiosensitization by increased apoptosis and cell senescence and by regulating intracellular reactive oxygen species. CONCLUSION: These results suggest that p53 status has an important role in the tumor-inhibiting and radiosensitizing effects of decreasing Prx I. Both Prx I and p53 may be powerful prognosticators for lung cancer.

Chen, Y., M. Pacyna-Gengelbach, et al. (2007). "5-Bromodeoxyuridine induced differentiation of a human small cell lung cancer cell line is associated with alteration of gene expression." Biochem Biophys Res Commun **353**(3): 559-64.

Small cell lung cancer (SCLC) appears to arise from neuroendocrine cells with the potential to differentiate into a variety of lung epithelial cell lineages. In order to investigate molecular events underlying the cell type transition in SCLC, we treated a SCLC cell line H526 with a differentiation inducing agent 5-bromodeoxyuridine (BrdU). The treatment led to a dramatic conversion from suspension cells to adherent cells exhibiting an epithelioid phenotype, which remarkably reduced the ability of colony formation in soft agar and suppressed the tumor growth rate in nude mice. The phenotypic transition was consistent with upregulation of surfactant protein C (SFTPC), thyroid transcription factor 1 (TTF-1), Connexin 26 (Cx26), insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1), as well as homeobox genes LAGY, PITX1, and HOXB2. Our data suggest that BrdU induced cell differentiation could be linked to the development of a less aggressively phenotype in small cell lung cancer.

Chen, Y., J. Sen, et al. (2009). "Novel cationic lipid that delivers siRNA and enhances therapeutic effect in lung cancer cells." Mol Pharm **6**(3): 696-705.

We have developed lipid-polycation-DNA (LPD) nanoparticles containing DOTAP and targeted with polyethylene glycol (PEG) tethered with anisamide (AA) to specifically deliver siRNA to H460 human lung carcinoma cells which express the sigma receptor. A novel non-glycerol based cationic lipid which contains both a guanidinium and a lysine residue as the cationic headgroup, i.e. DSGLA, downregulated pERK more efficiently in H460 cells than DOTAP. As demonstrated by using fluorescently labeled siRNA, LPD-PEG-AA prepared with DSGLA efficiently delivered siRNA to the cytoplasm of the H460 cells. Although the siRNA delivered by LPD-PEG-AA containing either DOTAP or DSGLA could effectively silence EGFR expression, a synergistic cell killing effect in promoting cellular apoptosis was only observed with DSGLA. The fluorescently labeled siRNA was efficiently delivered into the cytoplasm of H460 xenograft tumor by the LPD-PEG-AA containing either DOTAP or DSGLA 4 h after intravenous injection. Three daily injections (0.6 mg/kg) of siRNA formulated in the LPD-PEG-AA containing either DOTAP or DSGLA could effectively silence the epidermal growth factor receptor (EGFR) in the tumor, but the formulation containing DSGLA could induce more cellular apoptosis. A significant improvement in tumor growth inhibition was observed after dosing with LPD-PEG-AA containing DSGLA. Thus, DSGLA served as both a formulation component as well as a therapeutic agent which synergistically enhanced the activity of siRNA.

Chen, Y. L., P. Y. Law, et al. (2004). "Inhibition of akt/protein kinase B signaling by naltrindole in small cell lung cancer cells." Cancer Res **64**(23): 8723-30.

The phosphatidylinositol 3-kinase-Akt/protein kinase B (PKB) survival signaling is very important for cancer cell survival and growth. Constitutively active phosphatidylinositol 3-kinase-Akt/PKB signaling in small cell lung cancer (SCLC) is a major factor for the survival of SCLC cells. Inhibitors of this signaling pathway would be potential antitumor agents, particularly for SCLC. Here we report that naltrindole, which has been used as a classic delta opioid antagonist, inhibited growth and induced apoptosis in the three characteristic SCLC cell lines, NCI-H69, NCI-H345, and NCI-H510. Naltrindole treatment reduced constitutive phosphorylation of Akt/PKB on serine 473 and threonine 308 in cells. We found that the levels of constitutive phosphorylation of Akt/PKB on serine 473 correlate with the sensitivity of the three cell lines to naltrindole treatment. Furthermore, naltrindole treatment not only reduced the phosphorylation of the Akt/PKB upstream kinase phosphoinositide-dependent kinase-1, but also its downstream effectors glycogen synthase kinase-3beta and the Forkhead transcription factors AFX and FKHR. DNA array analysis of 205 apoptosis-related genes indicated that some Akt/PKB-dependent genes were either up- or down-regulated by naltrindole. Flow cytometric and microscopic analyses clearly showed that naltrindole induced apoptosis in SCLC cells. RNA interference experiments confirmed that naltrindole-induced cell death was associated with the Akt/PKB survival pathway. Together, these results show that naltrindole is a new inhibitor of the Akt/PKB signaling pathway, suggesting that naltrindole could be a potential lead for the development of a new type of inhibitors that target the constitutively active Akt/PKB signaling-dependent SCLC cells.

Choi, N., D. S. Son, et al. (2005). "RASSF1A is not appropriate as an early detection marker or a prognostic marker for non-small cell lung cancer." Int J Cancer **115**(4): 575-81.

Aberrant methylation of several tumor suppressor genes often occurs during the pathogenesis of lung cancer. RASSF1A is one of the tumor suppressor genes, and it is frequently inactivated by hypermethylation of its promoter region in a variety of human cancers, including lung cancer. It has recently

been suggested that RASSF1A methylation was frequently observed in poorly differentiated tumors, and that it was correlated with adverse survival in lung adenocarcinoma (Tomizawa Y, et al., Clin Cancer Res 2002;8:2362-8). In this study, we investigated the pathogenetic and clinicopathologic significance of RASSF1A methylation for the development and/or progression of non small cell lung cancer (NSCLC). We examined 116 cases of NSCLC for the methylation status of RASSF1A. Methylation-specific analysis demonstrated that 40.5% (47 of 116) of the cases were methylated at the CpG sites in the promoter. Methylation of RASSF1A was associated with cellular differentiation ($p = 0.0244$) and it was related to survival ($p = 0.0276$). However, there was no association between RASSF1A methylation and the individual clinicopathologic features: TNM stage ($p > 0.1$), recurrence ($p > 0.1$), lymphatic permeation ($p > 0.1$) and smoking duration time ($p > 0.1$). Furthermore, we analyzed RASSF1A's probability as a prognostic marker by using stepwise Cox proportional hazard regression testing. As a result, the stage proved to be the most important factor ($p = 0.0089$), more than any other factors such as age, gender, cell type, methylation status, differentiation, smoking duration time, tumor size and lymph node permeation. There was no other significant factor other than stage and age. These results show that epigenetic inactivation of RASSF1A cannot be a prognostic marker of NSCLC.

Cohen, V., J. S. Agulnik, et al. (2006). "Evaluation of denaturing high-performance liquid chromatography as a rapid detection method for identification of epidermal growth factor receptor mutations in nonsmall-cell lung cancer." Cancer **107**(12): 2858-65.

BACKGROUND: Somatic mutations of the epidermal growth factor receptor (EGFR) gene in nonsmall-cell lung cancer (NSCLC) may predict responsiveness to tyrosine kinase inhibitors. These mutations are commonly identified using DNA sequencing methods. Although considered the gold standard, this approach is time-consuming. In addition, this approach requires large diagnostic specimens and a high ratio of tumor-to-normal-tissue DNA for optimal results. The use of denaturing high-performance liquid chromatography (dHPLC) as a method to screen for the 2 predominant EGFR mutations is reported. METHODS: Clinical specimens from 104 NSCLC patients were analyzed for EGFR mutations in exons 19 and 21. After DNA extraction and polymerase chain reaction (PCR), both direct sequencing and dHPLC were performed and the results were compared. RESULTS: Sequencing revealed a total of 7 mutations: 3 deletion mutations in exon 19 and 4 missense mutations in exon 21. dHPLC showed the presence of genomic alterations in 23 samples, including the 7 identified by sequencing plus 16 additional samples (10 in exon 19 and 1 in exon 21). dHPLC fractions were isolated, reamplified, and sequenced to confirm the results. In serial dilution studies, dHPLC was able to detect mutations in samples containing as little as 1.6% to 6.25% mutated DNA, whereas direct sequencing required at least 30%. CONCLUSIONS: dHPLC is an efficient and more sensitive method for screening for genomic alterations in exons 19 and 21 of the EGFR gene compared with direct sequence analysis. These data suggest that dHPLC should be implemented as a screening tool for detection of EGFR mutations.

Dahse, R., A. Berndt, et al. (2008). "PCR-based testing for therapy-related EGFR mutations in patients with non-small cell lung cancer." Anticancer Res **28**(4B): 2265-70.

BACKGROUND: In patients with non-small cell lung cancer (NSCLC), mutations in the epidermal growth factor receptor gene (EGFR) have been associated with improved response to tyrosine kinase inhibitors. Two hotspot mutations located in exon 19 and exon 21 account for about 90% of all EGFR mutations. MATERIALS AND METHODS: We designed a Bi-PASA (bidirectional PCR amplification of specific alleles) assay to detect the most common exon 19 deletion (codons 746-750) and an allele-specific PCR for the L858R mutation in exon 21. To validate the assays for use in clinical diagnostics, 35 lung adenocarcinoma samples were analyzed. RESULTS: Both assays provided the predicted amplification pattern for normal and mutant genotypes with high specificity and sensitivity. In serial dilution experiments, the mutant alleles were detectable in mixed samples with an at least 6-fold excess of normal DNA. Three exon 19 deletions were identified in the tumor samples. CONCLUSION: Both assays are fast and easy to perform in any routine PCR laboratory with no special equipment other than thermocyclers. They provide sensitive and cost effective initial EGFR testing for identifying lung cancer patients who might clinically benefit from tyrosine kinase inhibitors.

d'Amato, T. A., R. J. Landreneau, et al. (2007). "Chemotherapy resistance and oncogene expression in non-small cell lung cancer." J Thorac Cardiovasc Surg **133**(2): 352-63.

OBJECTIVES: Empiric chemotherapy for patients with non-small cell lung cancer who have undergone resection is recommended without knowledge of the tumor's specific biologic characteristics, and many patients may not benefit. In vitro chemotherapy resistance is associated with

clinical unresponsiveness in some tumors, and in lung cancer, chemotherapy resistance is prevalent. Multiple-agent chemotherapy resistance and association of chemotherapy resistance with molecular markers are described. METHODS: Chemotherapy resistance to doublets--carboplatin and paclitaxel, cisplatin and navelbline, cisplatin and docetaxel, and cisplatin and gemcitabine--was analyzed in 4571 non-small cell lung cancer tumors with the extreme drug resistance assay. Chemotherapy resistance is defined as follows: extreme drug resistance, 1 SD above the median chemotherapy resistance; intermediate drug resistance, between the median and extreme drug resistances; and low drug resistance, 1 SD below the median. Chemotherapy resistance was compared with DNA ploidy measured by flow cytometry, and markers p53 and epithelial growth factor receptor were assayed by immunohistochemistry. RESULTS: Tumors with extreme or intermediate drug resistance were noted in 30% to carboplatin-paclitaxel, in 24% to cisplatin-navelbline, in 42% to cisplatin-gemcitabine, and in 27% to cisplatin-docetaxel. Extreme or intermediate drug resistance to at least one drug occurred in 74% to carboplatin-paclitaxel, in 68% to cisplatin-navelbline, in 88% to cisplatin-gemcitabine, and in 68% to cisplatin-docetaxel. More intermediate plus extreme chemotherapy resistances occurred in aneuploid tumors to etoposide (53% vs 36%, P = .0002) and topotecan (48% vs 36%, P = .0094), with less intermediate or extreme chemotherapy resistance to gemcitabine (88% vs 81%, P = .0345). p53-Positive tumors had more intermediate or extreme resistance to etoposide (57% vs 44%, P = .0009) and doxorubicin (73% vs. 58%, P = .0324) and less intermediate or extreme resistance to cisplatin (44% vs 54%, P = .0125), to carboplatin (47% vs 57%, P = .0129), to taxol (47% vs 57%, P = .0056), and to gemcitabine (78% vs 87%, P = .0108). Fewer epithelial growth factor receptor-positive tumors were extremely drug resistant to cisplatin (13% vs 26%, P = .0074) and carboplatin (13% v. 30%, P = .0008). CONCLUSIONS: Multi-drug chemotherapy resistance in non-small cell lung cancer tumor cultures is common, and associations between molecular markers and in vitro chemotherapy resistance are noted. Clinical validation through integration of such testing into clinical trials seems warranted.

Daniele, L., L. Macri, et al. (2007). "Predicting gefitinib responsiveness in lung cancer by fluorescence in situ hybridization/chromogenic in situ hybridization analysis of EGFR and HER2 in biopsy and cytology specimens." Mol Cancer Ther **6**(4): 1223-9.

In non-small cell lung cancer (NSCLC), epidermal growth factor receptor (EGFR) mutational analysis is an excellent predictor of responsiveness to treatment with tyrosine kinase inhibitors, such as gefitinib. In up to 80% of NSCLCs, cytologic samples or endoscopic biopsies are the only specimens available for molecular analysis, but PCR amplification of DNA from small fixed and paraffin-embedded samples may create artifactual mutations. Fluorescence in situ hybridization (FISH) of EGFR and HER2 has been proposed as an alternative method of analysis. This project aimed to determine the optimal scoring method for FISH or chromogenic in situ hybridization (CISH) assays when analyzing small NSCLC samples to predict response. FISH or CISH analysis of EGFR and HER2 genes was done on 42 small samples derived from NSCLC patients treated with gefitinib. EGFR mutational analysis was done after quantity and quality controls of DNA. In seven of seven cases, a balanced increase in EGFR gene and chromosome 7 number was found to correlate with the presence of specific EGFR mutations. In addition, seven of seven cases with balanced EGFR/HER2 polysomy and two of three cases with balanced EGFR/HER2 trisomy responded to gefitinib (75% of responders). Instead, the EGFR mutations predicted only 7 of 12 (58%) of gefitinib-responsive patients. When only endoscopic biopsies or cytologic specimens are available, we propose using FISH/CISH for EGFR and HER2 as the test of choice for selecting patients for treatment with gefitinib and to consider as negative predictive factor the absence of EGFR/HER2 gene gain.

de La Motte Rouge, T., L. Galluzzi, et al. (2007). "A novel epidermal growth factor receptor inhibitor promotes apoptosis in non-small cell lung cancer cells resistant to erlotinib." Cancer Res **67**(13): 6253-62.

Non-small cell lung cancer (NSCLC) with activating mutations in the epidermal growth factor receptor (EGFR) responds to EGFR tyrosine kinase inhibitors such as erlotinib. However, secondary somatic EGFR mutations (e.g., T790M) confer resistance to erlotinib. BMS-690514, a novel panHER/vascular endothelial growth factor receptor (VEGFR) inhibitor described here, exerted antiproliferative and proapoptotic effects on NSCLC cell lines, with prominent efficacy on H1975 cells expressing the T790M mutation. In this model, BMS-690514 induced a G(1) cell cycle arrest, as well as ultrastructural hallmarks of apoptosis, mitochondrial release of cytochrome c, and activation of caspases involved in the intrinsic (e.g., caspase-2, caspase-3, caspase-7, and caspase-9), but not in the extrinsic (e.g., caspase-8), pathway. Caspase inhibition conferred partial protection against BMS-690514 cytotoxicity, pointing to the involvement of both caspase-dependent and caspase-independent effector

mechanisms. Transcriptome analyses revealed the up-regulation of proapoptotic (e.g., Bim, Puma) and cell cycle inhibitory (e.g., p27(Kip1), p57(Kip2)) factors, as well as the down-regulation of antiapoptotic (e.g., Mcl1), heat shock (e.g., HSP40, HSP70, HSP90), and cell cycle promoting [e.g., cyclins B1, D1, and D3; cyclin-dependent kinase 1 (CDK1); MCM family proteins; proliferating cell nuclear antigen (PCNA)] proteins. BMS-690514-induced death of H1975 cells was modified in a unique fashion by a panel of small interfering RNAs targeting apoptosis modulators. Down-regulation of components of the nuclear factor-kappaB survival pathway (e.g., p65, Nemo/IKK gamma, TAB2) sensitized cells to BMS-690514, whereas knockdown of proapoptotic factors (e.g., Puma, Bax, Bak, caspase-2, etc.) and DNA damage-related proteins (e.g., ERCC1, hTERT) exerted cytoprotective effects. BMS-690514 is a new pan-HER/VEGFR inhibitor that may become an alternative to erlotinib for the treatment of NSCLC.

de las Penas, R., M. Sanchez-Ronco, et al. (2006). "Polymorphisms in DNA repair genes modulate survival in cisplatin/gemcitabine-treated non-small-cell lung cancer patients." Ann Oncol **17**(4): 668-75.

BACKGROUND: Impaired DNA repair capacity may favorably affect survival in cisplatin/gemcitabine-treated non-small-cell lung cancer (NSCLC) patients. We investigated the association of survival with genetic polymorphisms in X-ray repair cross-complementing group 1 and group 3 (XRCC3), xeroderma pigmentosum group D (XPD), excision repair cross-complementing group 1, ligase IV, ribonucleotide reductase, TP53, cyclooxygenase-2, interleukin-6, peroxisome proliferator-activated receptor gamma, epidermal growth factor, methylene-tetra-hydrofolate reductase and methionine synthase. PATIENTS AND METHODS: One hundred and thirty-five stage IV or IIIB (with malignant pleural effusion) NSCLC patients treated with cisplatin/gemcitabine from different hospitals of the Spanish Lung Cancer Group were genotyped for 14 different polymorphisms in 13 genes. Polymorphisms were detected by the TaqMan method, using genomic DNA extracted from baseline blood samples. RESULTS: Median survival was significantly increased in patients harboring XRCC3 241 MetMet: 16 months versus 10 months for patients with ThrMet and 14 months for those with ThrThr (P = 0.01). The risk of death ratio was significantly lower for MetMet than for ThrMet patients (hazard ratio, 0.43; P = 0.01). In the multivariate Cox model, XRCC3 241 remained an independent prognostic factor (hazard ratio: XRCC3 241 MetMet, 0.44; P = 0.01), and XPD 751 and XRCC1 399 also emerged as significant prognostic factors (hazard ratios: XPD 751 LysGln, 0.46, P = 0.03; XRCC1 399 ArgGln, 0.61, P = 0.04). No other association was observed between genotype and survival. CONCLUSION: XRCC3 241 MetMet is an independent determinant of favorable survival in NSCLC patients treated with cisplatin/gemcitabine. A simple molecular assay to determine the XRCC3 241 genotype can be useful for customizing chemotherapy.

Deng, W. G., G. Jayachandran, et al. (2007). "Tumor-specific activation of human telomerase reverses transcriptase promoter activity by activating enhancer-binding protein-2beta in human lung cancer cells." J Biol Chem **282**(36): 26460-70.

The up-regulated expression and telomerase activity of human telomerase reverse transcriptase (hTERT) are hallmarks of tumorigenesis. The hTERT promoter has been shown to promote hTERT gene expression selectively in tumor cells but not in normal cells. However, little is known about how tumor cells differentially activate hTERT transcription and induce telomerase activity. In this study, we identified activating enhancer-binding protein-2beta (AP-2beta) as a novel transcription factor that specifically binds to and activates the hTERT promoter in human lung cancer cells. AP-2beta was detected in hTERT promoter DNA-protein complexes formed in nuclear extracts prepared only from lung cancer cells but not from normal cells. We verified the tumor-specific binding activity of AP-2beta for the hTERT promoter in vitro and in vivo and detected high expression levels of AP-2beta in lung cancer cells. We found that ectopic expression of AP-2beta reactivated hTERT promoter-driven reporter green fluorescent protein (GFP) gene and endogenous hTERT gene expression in normal cells, enhanced GFP gene expression in lung cancer cells, and prolonged the life span of primary lung bronchial epithelial cells. Furthermore, we found that inhibition of endogenous AP-2beta expression by AP-2beta gene-specific small interfering RNAs effectively attenuated hTERT promoter-driven GFP expression, suppressed telomerase activity, accelerated telomere shortening, and inhibited tumor cell growth by induction of apoptosis in lung cancer cells. Our results demonstrate the tumor-specific activation of the hTERT promoter by AP-2beta and imply the potential of AP-2beta as a novel tumor marker or a cancer therapeutic target.

Denlinger, C. E., M. D. Keller, et al. (2004). "Combined proteasome and histone deacetylase inhibition in non-small cell lung cancer." J Thorac Cardiovasc Surg **127**(4): 1078-86.

OBJECTIVE: Inhibitors of histone deacetylases are potent inducers of cell-cycle arrest and apoptosis in certain malignancies. We have previously demonstrated that chemotherapy activates

the antiapoptotic transcription factor nuclear factor kappa B in non-small cell lung cancer and fails to induce significant levels of apoptosis. We hypothesize that nuclear factor kappa B inhibition with the proteasome inhibitor bortezomib (formerly known as PS-341) will sensitize non-small cell lung cancer cells to histone deacetylase inhibitor-mediated apoptosis. METHODS: Tumorigenic non-small cell lung cancer cells (A549, H358, and H460) were treated with bortezomib, followed by the histone deactylase inhibitor sodium butyrate. After treatment, nuclear factor kappa B transcriptional activity was measured by using a luciferase reporter assay and transcription of the nuclear factor kappa B-dependent gene IL8. Apoptosis was determined on the basis of caspase-3 activation and DNA fragmentation. Western blot analyses for the cell-cycle regulatory proteins p21 and p53 were performed, and cell-cycle alterations were determined by means of FACS analysis. Experiments were performed in triplicate, and statistical significance was determined by using unpaired t tests. RESULTS: Butyrate increased nuclear factor kappa B transcriptional activity 4-fold relative to that seen in control cells (P =.05) in all non-small cell lung cancer cell lines. Treatment with bortezomib reduced butyrate-induced activation of nuclear factor kappa B to baseline levels. The proteins p21 and p53 were stabilized after treatment with bortezomib, correlating with a G(2)/M cell-cycle arrest. Treatment with butyrate alone resulted in minimal apoptosis, but combined histone deacetylase and proteasome inhibition increased apoptosis 3- to 4-fold (P =.02). CONCLUSIONS: Combined molecular targeting of histone deacteylases and proteasomes synergistically induced apoptosis in non-small cell lung cancer. Pharmacologic nuclear factor kappa B suppression through proteasome inhibition, followed by treatment with histone deacetylase inhibitors, might represent a novel treatment strategy for patients with non-small cell lung cancer.

Denlinger, C. E., B. K. Rundall, et al. (2004). "Proteasome inhibition sensitizes non-small cell lung cancer to histone deacetylase inhibitor-induced apoptosis through the generation of reactive oxygen species." J Thorac Cardiovasc Surg **128**(5): 740-8.

OBJECTIVES: The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces apoptosis in some malignancies through mitochondrial injury and generation of reactive oxygen species. Histone deacetylase inhibitors also activate the antiapoptotic transcription factor nuclear factor kappaB. We hypothesize that proteasome inhibition with bortezomib (Velcade; Millennium Pharmaceuticals, Inc, Cambridge, Mass)will inhibit nuclear factor kappaB activation, enhance suberoylanilide hydroxamic acid-induced mitochondrial injury, and sensitize non-small cell lung cancer cells to apoptosis. METHODS: Four tumorigenic non-small cell lung cancer cell lines were treated with nothing, suberoylanilide hydroxamic acid, bortezomib, or both drugs. Nuclear factor kappaB-dependent transcription was determined by reporter gene assays and endogenous interleukin 8 transcription. Reactive oxygen species were quantified by using the fluorophore H 2 DCFDA. Cell viability was determined on the basis of clonogenic survival, and apoptosis was measured by quantifying caspase-3 activity and DNA fragmentation. Apoptosis and cell-survival assays were repeated in similarly treated cells incubated in the presence or absence of N-acetyl cysteine. Statistical significance was determined by means of analysis of variance. RESULTS: Suberoylanilide hydroxamic acid significantly enhanced interleukin 8 and nuclear factor kappaB-dependent reporter gene transcription, and these effects were inhibited by bortezomib (P < or = .01). Combined treatment with suberoylanilide hydroxamic acid and bortezomib induced greater reactive oxygen species generation, more apoptosis (P < or = .02), and more cell death (P < or = .001) than either drug alone. N-acetyl cysteine diminished the induction of apoptosis and enhanced cell survival (P < or = .04). CONCLUSIONS: Suberoylanilide hydroxamic acid and bortezomib synergistically induce reactive oxygen species generation in non-small cell lung cancer, and this plays a critical role in the induction of apoptosis after treatment. Combined treatment with suberoylanilide hydroxamic acid and bortezomib might be an effective treatment strategy for non-small cell lung cancer.

Denlinger, C. E., B. K. Rundall, et al. (2005). "Inhibition of phosphatidylinositol 3-kinase/Akt and histone deacetylase activity induces apoptosis in non-small cell lung cancer in vitro and in vivo." J Thorac Cardiovasc Surg **130**(5): 1422-9.

OBJECTIVE: Resistance to histone deacetylase inhibitors in non-small cell lung cancer is mediated in part through activation of nuclear factor-kappaB through a phosphatidylinositol 3-kinase/Akt-dependent pathway. We hypothesize that inhibition of phosphatidylinositol 3-kinase/Akt will sensitize non-small cell lung cancer cells to histone deacetylase inhibitor-induced apoptosis. METHODS: Tumorigenic non-small cell lung cancer cell lines H157, H358, H460, and A549 were treated with nothing, the histone deacetylase inhibitor butyrate, the phosphatidylinositol 3-kinase/Akt inhibitor LY294002, or both compounds. Nuclear factor-kappaB activity was assessed by reporter gene assays and reverse transcriptase-polymerase chain reaction of

the nuclear factor-kappaB dependent genes cIAP-2, Bfl/A1, and MnSOD. Whole cell extracts were immunoblotted for phospho-Akt, Akt, and phospho-ser/thr-Akt substrate. Cell death and apoptosis were measured by crystal violet staining, caspase-3 activity, and DNA fragmentation. A549 non-small cell lung cancer xenografts were created in athymic nude mice, and tumor growth was assessed after treatments as noted above. Explanted tumors underwent terminal deoxynucleotide transferase-mediated dUTP nick-end labeling and Western blot analyses for apoptosis assessment and drug target validation, respectively. RESULTS: Butyrate activated nuclear factor-kappaB-dependent transcription, and LY294002 abrogated this effect. Combined treatment induced more apoptosis and cell death in vitro compared with either drug alone as measured by caspase-3, DNA fragmentation, and clonogenic survival. Combined butyrate and LY294002 was tumoristatic in vivo, but all other xenografts grew. This decreased tumor growth correlated with more apoptosis in the xenografts treated with combined therapy. Tumor levels of phospho-Akt and acetylated histone H3 were decreased and increased, respectively, in xenografts treated with combined therapy. CONCLUSIONS: Combined histone deacetylase inhibitor and phosphatidylinositol 3-kinase/Akt pathway inhibition sensitized non-small cell lung cancer xenografts to apoptosis. Further investigations of this combined therapy are warranted as new pharmacologic phosphatidylinositol 3-kinase/Akt pathway inhibitors are developed.

Denlinger, C. E., B. K. Rundall, et al. (2004). "Proteasome inhibition sensitizes non-small-cell lung cancer to gemcitabine-induced apoptosis." Ann Thorac Surg **78**(4): 1207-14; discussion 1207-14.

BACKGROUND: My colleagues and I have previously shown that chemotherapy activates the antiapoptotic transcription factor nuclear factor (NF)-kappaB in non-small-cell lung cancer (NSCLC). We hypothesized that inhibition of NF-kappaB by using the proteasome inhibitor bortezomib (Velcade) would sensitize NSCLC to gemcitabine-induced apoptosis. METHODS: Tumorigenic NSCLC cell lines (H157 and A549) were treated with nothing, gemcitabine, bortezomib, or both compounds. NF-kappaB activity was determined by nuclear p65 protein levels, electrophoretic mobility shift assays, and reverse transcription-polymerase chain reaction of the NF-kappaB-regulated genes interleukin-8, c-IAP2, and Bcl-xL. The p21 and p53 protein levels were determined in similarly treated cells. Cell-cycle dysregulation was assessed by fluorescence-activated cell sorting analysis. Cell death and apoptosis were quantified by clonogenic assays, caspase-3 activation, and DNA fragmentation. NSCLC A549 xenografts were generated and treated as noted previously. Tumor growth was assessed over a 4-week treatment period. Statistical analysis was performed with analysis of variance. RESULTS: Gemcitabine enhanced nuclear p65 levels, NF-kappaB binding to DNA, and transcription of all NF-kappaB-regulated genes. Bortezomib inhibited each of these effects. Combined gemcitabine and bortezomib enhanced p21 and p53 expression and induced S-phase and G2/M cell-cycle arrests, respectively. Combined treatment killed 80% of the NSCLC cells and induced apoptosis, as determined by caspase-3 activation ($p = 0.05$) and DNA fragmentation ($p = 0.02$). NSCLC xenografts treated with combination therapy grew significantly slower than xenografts treated with gemcitabine alone ($p = 0.02$). CONCLUSIONS: Bortezomib inhibits gemcitabine-induced activation of NF-kappaB and sensitizes NSCLC to death in vitro and in vivo. This combined treatment strategy warrants further investigation and may represent a reasonable treatment strategy for select patients with NSCLC given the current clinical availability of both drugs.

Dey, A., E. T. Wong, et al. (2007). "Nutlin-3 inhibits the NFkappaB pathway in a p53-dependent manner: implications in lung cancer therapy." Cell Cycle **6**(17): 2178-85.

Nutlins were identified as the first potent and specific small molecule Mdm2 antagonists that inhibit the p53-Mdm2 interaction. We show in this study that Nutlin-3 can downregulate TNFalpha induced activation of the NFkappaB reporter in lung cancer cells. Activation of p53 dependent transcription is not compromised when Nutlin-3 is combined with TNFalpha. Instead, this combination treatment decreases cell viability in a p53 dependent manner. We show that Nutlin-3 strikingly inhibits the protein expression of NFkappaB target genes ICAM-1 and MCP-1 while other targets like Bcl-xL and FLIP are not affected, thereby suggesting that the inhibition is promoter specific. This inhibition of ICAM-1 and MCP-1 by Nutlin-3 is again dependent on the p53 status in cells. Furthermore, we show that Nutlin-3 strongly inhibits protein expression of ICAM-1 and MCP-1 induced by IL1, another NFkappaB activating stimuli. Nutlin-3 does not inhibit Akt phosphorylation, IkappaB alpha phosphorylation, IkappaB alpha degradation, p65 modification or p65 DNA binding in the cell lines tested. This study suggests the potential of Nutlin-3 as a bitargeted anti-cancer drug by simultaneously causing p53 activation and NFkappaB suppression. It also suggests that Nutlin-3 could be evaluated for treatment of lung cancer as a single agent or in combination therapy by targeting its effect on ICAM-1 and MCP-1 which are known to be

critical for cancer cell invasion, thereby downregulating tumor formation and metastasis. This study also suggests biomarkers of response for evaluation of Nutlin-3 in the clinic.

Dong, A. Q., M. J. Kong, et al. (2007). "Down-regulation of IGF-IR using small, interfering, hairpin RNA (siRNA) inhibits growth of human lung cancer cell line A549 in vitro and in nude mice." Cell Biol Int **31**(5): 500-7.

Type I insulin-like growth factor receptor (IGF-IR), which is frequently overexpressed in a variety of human cancers including lung cancer, mediates cancer cell proliferation and tumor growth. In this study, we used a human U6 promoter-driven DNA-template approach to induce hairpin RNA (hpRNA)-triggered RNAi to silence IGF-IR gene expression in the human lung cancer cell line A549, and then evaluate its effects on apoptosis, apoptosis-related gene expression, and the growth of tumor cells in vitro and in nude mice. IGF-IR expression levels were found to markedly decrease in cells transfected with a plasmid expressing hairpin siRNA for IGF-IR (by more than 78.9%). Down-regulation of IGR-IR concomitantly accompanied reduction of bcl-2 as well as pERK and pAkt levels, activation of caspase-3, apoptosis and growth inhibition of A549 cells in vitro. Direct intratumoral injections of plasmid DNA expressing hpRNA for IGF-IR significantly regressed pre-established tumors in nude mice. Our results support the therapeutic potential of RNAi as a method for gene therapy in treating lung cancer.

Dongiovanni, D., L. Daniele, et al. (2008). "Gefitinib (ZD1839): therapy in selected patients with non-small cell lung cancer (NSCLC)?" Lung Cancer **61**(1): 73-81.

PURPOSE: To evaluate response rate, toxicity and epidermal growth factor (EGFR) mutations and gene copy number as outcome predictive factors in Italian patients with non-small cell lung cancer (NSCLC) treated with gefitinib (Iressa) in an expanded access program (EAP). PATIENTS AND METHODS: A total of 137 patients with advanced NSCLC received gefitinib as first line treatment or after failure of chemotherapy. In 43 cases, tissue specimens were available for EGFR status evaluation: immunohistochemical (IHC) for EGFR, fluorescence in situ hybridisation (FISH) or Chromogenic in situ hybridisation (CISH)-(ISH) analysis for EGFR and HER2 gene copy number, and PCR-DNA sequencing for mutational analysis of EGFR were performed. RESULTS: In the study population, response rate (PR) was 13%; disease stabilization (DS) 26%; overall disease control rate 39%; median survival 6.3 months and time to progression 2.7 months. Toxicity was mild (G3 skin toxicity in 3% and G3 liver toxicity in 4% of patients). An EGFR-mutation was detected in 9/43 patients: Eight deletions in exon 19 and 1 missense mutation in exon 21. Increased gene copy number for EGFR and/or HER2 was detected in 17/43 patients. Response rate was significantly higher in women, non-smokers, in mutation carriers than in wild type carriers, in EGFR-trisomy/polysomy carriers and HER2-trisomy/polysomy carriers. CONCLUSIONS: In this study, response rate and toxicity to gefitinib treatment were consistent with previously reported data for whites. Female gender, absence of smoking history, EGFR-mutations, EGFR and HER2-polysomy were significantly associated with response to gefitinib therapy in NSCLC patients.

Droemann, D., D. Albrecht, et al. (2005). "Human lung cancer cells express functionally active Toll-like receptor 9." Respir Res **6**: 1.

BACKGROUND: CpG-oligonucleotides (CpG-ODN), which induce signaling through Toll-like receptor 9 (TLR9), are currently under investigation as adjuvants in therapy against infections and cancer. CpG-ODN function as Th-1 adjuvants and are able to activate dendritic cells. In humans TLR9 has been described to be strongly expressed in B-lymphocytes, monocytes, plasmacytoid dendritic cells and at low levels in human respiratory cells. We determined whether a direct interaction of bacterial DNA with the tumor cells themselves is possible and investigated the expression and function of TLR9 in human malignant solid tumors and cell lines. TLR9 expression by malignant tumor cells, would affect treatment approaches using CpG-ODN on the one hand, and, on the other hand, provide additional novel information about the role of tumor cells in tumor-immunology. METHODS: The expression of TLR9 in HOPE-fixed non-small lung cancer, non-malignant tissue and tumor cell lines was assessed using immunohistochemistry, confocal microscopy, in situ hybridization, RT-PCR and DNA-sequencing. Apoptosis and chemokine expression was detected by FACS analysis and the Bio-Plex system. RESULTS: We found high TLR9 signal intensities in the cytoplasm of tumor cells in the majority of lung cancer specimens as well as in all tested tumor cell lines. In contrast to this non-malignant lung tissues showed only sporadically weak expression. Stimulation of HeLa and A549 cells with CpG-ODN induced secretion of monocyte chemoattractant protein-1 and reduction of spontaneous and tumor necrosis factor-alpha induced apoptosis. CONCLUSIONS: Here we show that TLR9 is expressed in a selection of human lung cancer tissues and various tumor cell lines. The expression of

functionally active TLR9 in human malignant tumors might affect treatment approaches using CpG-ODN and shows that malignant cells can be regarded as active players in tumor-immunology.

Dubey, S., P. Stephenson, et al. (2006). "EGFR dinucleotide repeat polymorphism as a prognostic indicator in non-small cell lung cancer." J Thorac Oncol **1**(5): 406-12.

BACKGROUND: The epidermal growth factor receptor (EGFR) has been implicated in tumor growth and progression. Intron 1 of the EGFR gene contains a polymorphic simple sequence repeat (SSR) of 14 to 21 CA dinucleotides, the length of which correlates inversely with the level of EGFR transcription. The authors hypothesized that a shorter length of tumor SSR would be associated with poorer survival in patients with non-small cell lung cancer (NSCLC). METHODS: Patients enrolled in Eastern Cooperative Oncology Group E3590 (a randomized, prospective trial of adjuvant therapy following resection of stages II and IIIa NSCLC) were randomized to radiation or radiation plus chemotherapy. Genomic DNA extracted from resected tumors was amplified for EGFR intron 1 by polymerase chain reaction and sequenced in a 3730XL DNA analyzer. RESULTS: One hundred fifty-seven primary tumors were sequenced, 106 (68%) of which were heterozygous for intron 1. The most common genotypes were allele lengths of 17/19 dinucleotides (17.8%), 17/18 (11.4%), and 19/19 (11.4%). Allele status (homozygous versus heterozygous) did not correlate with race, gender, weight, performance status, histology, stage, or survival. Shorter allele length (< or =18 versus >18 CA dinucleotide repeats) was associated with squamous cell histology (p = 0.03). Allele sum of greater than 35 was associated with improved overall survival (log-rank p = 0.03, hazard ratio = 0.66). CONCLUSION: This is the first study to characterize the EGFR intron 1 SSR polymorphism in NSCLC. Tumors were most commonly heterozygous for SSR length. Squamous histology was associated with a shorter SSR. Longer sequences are associated with improved survival.

Dziadziuszko, R., S. E. Witta, et al. (2006). "Epidermal growth factor receptor messenger RNA expression, gene dosage, and gefitinib sensitivity in non-small cell lung cancer." Clin Cancer Res **12**(10): 3078-84.

PURPOSE: Epidermal growth factor receptor (EGFR) mRNA expression and EGFR gene dosage by quantitative PCR in tumor samples obtained from patients with gefitinib-treated non-small cell lung cancer were analyzed in order to determine the association with treatment outcome, clinical, and biological features [EGFR copy number by fluorescent in situ hybridization (FISH), EGFR tyrosine kinase mutations, and EGFR protein expression]. EXPERIMENTAL DESIGN: EGFR mRNA expression was measured by real-time quantitative reverse transcription-PCR in 64 patients, and EGFR gene dosage was analyzed by real-time quantitative PCR in 82 patients from paraffin-embedded specimens. RESULTS: EGFR mRNA expression was higher in responders to gefitinib as compared with nonresponders (P = 0.012). Patients with high EGFR mRNA expression (>5.01) had 43% response probability, whereas patients with low EGFR mRNA expression had 8% response probability (P = 0.006). Patients with high EGFR mRNA expression had longer median progression-free (5.3 versus 2.8 months, P = 0.028) but not overall survival (13.8 versus 10.9 months, P = 0.87). EGFR mRNA expression was higher in FISH-positive patients (P = 0.001) and in patients with positive EGFR immunostaining (P < 0.001) but not in patients with EGFR mutations (P = 0.19). EGFR gene dosage did not predict response (P = 0.54), progression-free (P = 0.73), or overall survival (P = 0.89). EGFR gene dosage was not associated with FISH positivity (P = 0.15), relative mRNA expression (P = 0.27), EGFR mutation status (P = 0.39), and EGFR protein expression (P = 0.35). CONCLUSION: EGFR mRNA expression is a predictive biomarker for response to gefitinib and to progression-free survival after gefitinib treatment. EGFR gene dosage is neither predictive for response nor progression-free nor overall survival.

Eberhard, D. A., G. Giaccone, et al. (2008). "Biomarkers of response to epidermal growth factor receptor inhibitors in Non-Small-Cell Lung Cancer Working Group: standardization for use in the clinical trial setting." J Clin Oncol **26**(6): 983-94.

The body of literature on the correlations between molecular assessments and patient outcomes after treatment with epidermal growth factor receptor (EGFR) inhibitors continues to grow. It will be important in the future to determine how to most effectively integrate molecular assays that assess the likelihood of therapeutic benefit into clinical practice. Although EGFR-targeted therapies such as erlotinib have been approved for use without molecular testing, immunohistochemistry, fluorescence in situ hybridization, and mutational analyses of the EGFR gene have all been proposed as candidates to help predict response or survival benefit from EGFR-targeted therapy in patients with non-small-cell lung cancer (NSCLC). Further prospective validation from ongoing randomized studies will be needed to fully determine which assays are best to help predict patient

outcome. In addition, it will be critical for these assays to undergo standardization before widespread clinical use. The Molecular Assays in NSCLC Working Group, under the sponsorship of Genentech Inc, Roche Pharmaceuticals, and OSI Pharmaceuticals, Inc, was convened to evaluate the available molecular assays for use in the clinical trial setting and provide recommendations for application and interpretation of these tests for future clinical trials. Recommendations of the Molecular Assays in NSCLC Working Group for the use of EGFR molecular assays are presented and include guidelines for tissue storage, handling, and processing. Recommendations for the standardization of molecular assays are also discussed.

Eberhard, D. A., B. E. Johnson, et al. (2005). "Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib." J Clin Oncol **23**(25): 5900-9.

PURPOSE: Epidermal growth factor receptor (EGFR) mutations have been associated with tumor response to treatment with single-agent EGFR inhibitors in patients with relapsed non-small-cell lung cancer (NSCLC). The implications of EGFR mutations in patients treated with EGFR inhibitors plus first-line chemotherapy are unknown. KRAS is frequently activated in NSCLC. The relationship of KRAS mutations to outcome after EGFR inhibitor treatment has not been described. PATIENTS AND METHODS: Previously untreated patients with advanced NSCLC in the phase III TRIBUTE study who were randomly assigned to carboplatin and paclitaxel with erlotinib or placebo were assessed for survival, response, and time to progression (TTP). EGFR exons 18 through 21 and KRAS exon 2 were sequenced in tumors from 274 patients. Outcomes were correlated with EGFR and KRAS mutations in retrospective subset analyses. RESULTS: EGFR mutations were detected in 13% of tumors and were associated with longer survival, irrespective of treatment ($P < .001$). Among erlotinib-treated patients, EGFR mutations were associated with improved response rate ($P < .05$) and there was a trend toward an erlotinib benefit on TTP ($P = .092$), but not improved survival ($P = .96$). KRAS mutations (21% of tumors) were associated with significantly decreased TTP and survival in erlotinib plus chemotherapy-treated patients. CONCLUSION: EGFR mutations may be a positive prognostic factor for survival in advanced NSCLC patients treated with chemotherapy with or without erlotinib, and may predict greater likelihood of response. Patients with KRAS-mutant NSCLC showed poorer clinical outcomes when treated with erlotinib and chemotherapy. Further studies are needed to confirm the findings of this retrospective subset analysis.

Elrod, H. A., Y. D. Lin, et al. (2007). "The alkylphospholipid perifosine induces apoptosis of human lung cancer cells requiring inhibition of Akt and activation of the extrinsic apoptotic pathway." Mol Cancer Ther **6**(7): 2029-38.

The Akt inhibitor, perifosine, is an alkylphospholipid exhibiting antitumor properties and is currently in phase II clinical trials for various types of cancer. The mechanisms by which perifosine exerts its antitumor effects, including the induction of apoptosis, are not well understood. The current study focused on the effects of perifosine on the induction of apoptosis and its underlying mechanisms in human non-small cell lung cancer (NSCLC) cells. Perifosine, at clinically achievable concentration ranges of 10 to 15 micromol/L, effectively inhibited the growth and induced apoptosis of NSCLC cells. Perifosine inhibited Akt phosphorylation and reduced the levels of total Akt. Importantly, enforced activation of Akt attenuated perifosine-induced apoptosis. These results indicate that Akt inhibition is necessary for perifosine-induced apoptosis. Despite the activation of both caspase-8 and caspase-9, perifosine strikingly induced the expression of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor, death receptor 5, and down-regulated cellular FLICE-inhibitory protein (c-FLIP), an endogenous inhibitor of the extrinsic apoptotic pathway, with limited modulatory effects on the expression of other genes including Bcl-2, Bcl-X(L), PUMA, and survivin. Silencing of either caspase-8 or death receptor 5 attenuated perifosine-induced apoptosis. Consistently, further down-regulation of c-FLIP expression with c-FLIP small interfering RNA sensitized cells to perifosine-induced apoptosis, whereas enforced overexpression of ectopic c-FLIP conferred resistance to perifosine. Collectively, these data indicate that activation of the extrinsic apoptotic pathway plays a critical role in perifosine-induced apoptosis. Moreover, perifosine cooperates with TRAIL to enhance the induction of apoptosis in human NSCLC cells, thus warranting future in vivo and clinical evaluation of perifosine in combination with TRAIL in the treatment of NSCLC.

Endo, K., H. Sasaki, et al. (2006). "Evaluation of the epidermal growth factor receptor gene mutation and copy number in non-small cell lung cancer with gefitinib therapy." Oncol Rep **16**(3): 533-41.

Several studies have suggested that epidermal growth factor receptor (EGFR) gene mutation, EGFR gene amplification, and some other biomarkers may be predictors of gefitinib sensitivity. We analyzed

EGFR mutation and EGFR copy number in 22 gefitinib-treated non-small cell lung cancer (NSCLC) cases and their relation to the survival of patients. We also studied 143 gefitinib-naive Japanese NSCLC cases. The erbB2 copy number was also studied in 59 gefitinib-naive NSCLC cases. In gefitinib-treated patients, the presence of EGFR mutation was associated with a higher response rate to gefitinib and a longer overall survival, but the increased EGFR gene copy number was not. In gefitinib-naive cases, EGFR mutation but not EGFR gene copy number was significantly correlated with gender, pathological subtypes, and smoking status. The erbB2 copy number was not significantly correlated with the EGFR mutation or EGFR copy number in 59 cases. In conclusion, EGFR mutation was a better predictor of clinical outcome in gefitinib-treated patients than the EGFR gene copy number.

Felip, E., M. Taron, et al. (2005). "Clinical significance of hypoxia-inducible factor-1a messenger RNA expression in locally advanced non-small-cell lung cancer after platinum agent and gemcitabine chemotherapy followed by surgery." Clin Lung Cancer **6**(5): 299-303.

Hypoxia-inducible factor-1a (HIF-1a) is a key regulator of the angiogenic cascade. This study analyzed HIF-1a messenger RNA expression levels using real-time quantitative polymerase chain reaction (PCR) in paraffin-embedded surgical specimens from 54 stage IIB-III patients with non-small-cell lung cancer (NSCLC) treated with induction platinum/gemcitabine followed by surgery between September 1998 and December 2002. Radiographic response was observed in 61% of patients. Median survival was 37.8 months. Forty-five patients with complete resection attained a 52-month median survival, whereas 8 patients with incomplete resection had a 12-month median survival, and 1 unresectable patient had a survival of 14 months. No significant differences were observed in overall survival (OS) or event-free survival (EFS) according to HIF-1a expression levels. Patients were divided into quartiles according to HIF-1a gene expression levels. Median EFS for the 13 patients in the lowest quartile has not been reached yet, whereas median EFS for the 13 patients in the top quartile was 9 months (P = 0.192). Similarly, median OS for the 13 patients in the lowest quartile has not been reached yet, whereas median OS for the 13 patients in the top quartile was 52 months (P = 0.297). The cisplatin/gemcitabine combination is highly active in neoadjuvant treatment. Hypoxia-inducible factor-1a expression levels analyzed by real-time quantitative PCR in surgery specimens after platinum/gemcitabine therapy do not correlate with the outcome of patients with stage II/III NSCLC.

Feng, Q., S. E. Hawes, et al. (2008). "DNA methylation in tumor and matched normal tissues from non-small cell lung cancer patients." Cancer Epidemiol Biomarkers Prev **17**(3): 645-54.

We used MethyLight assays to analyze DNA methylation status of 27 genes on 49 paired cancerous and noncancerous tissue samples from non-small cell lung cancer (NSCLC) patients who underwent surgical resection. Seven genes (RARB, BVES, CDKN2A, KCNH5, RASSF1, CDH13, and RUNX) were found to be methylated significantly more frequently in tumor tissues than in noncancerous tissues. Only methylation of CCND2 and APC was frequently detected in both cancerous and noncancerous tissues, supporting the hypothesis that the methylation of these two genes is a preneoplastic change and may be associated with tobacco smoking exposure. Methylation of any one of eight genes (RASSF1, DAPK1, BVES, CDH13, MGMT, KCNH5, RARB, or CDH1) was present in 80% of NSCLC tissues but only in 14% of noncancerous tissues. Detection of methylation of these genes in blood might have utility in monitoring and detecting tumor recurrence in early-stage NSCLC after curative surgical resection.

Fischer, J. R., U. Ohnmacht, et al. (2007). "Prognostic significance of RASSF1A promoter methylation on survival of non-small cell lung cancer patients treated with gemcitabine." Lung Cancer **56**(1): 115-23.

The epigenetic inactivation of genes plays an important role in lung cancer. We have investigated the methylation status of the promoter region of seven genes (APC1A, DAPK, FHIT, p14(ARF), p16(INK4a), RARbeta, RASSF1A) in serum DNA of NSCLC patients. The objective of our study was to reveal the influence of such alterations on overall survival. Blood samples were drawn pretherapeutically. Genomic DNA was purified from serum, treated with sodium bisulfite and hypermethylation was detected by a nested methylation-specific PCR in a group of 92 patients with histologically confirmed stage IIIB and IV NSCLC. All patients received gemcitabine first-line alone or in combination with other drugs. The vast majority (n=87) showed at least one epigenetic alteration. The methylation frequencies of individual genes varied between 25.9 and 47.3%. The hypermethylation status of none of the genes had a significant influence on median overall survival of the total population. In contrast, patients with a methylated RASSF1A gene who showed a partial response survived significantly longer (33.6+/-10.4 month) compared to those with a wild-type allele (12.9+/-4.7 month, P=0.0045). This effect became

even more pronounced in combination with p14(ARF) (P=0.0004). This difference was not seen in patients with stable or progressive disease. A multivariate analysis confirmed that RASSF1A methylation was an independent prognostic factor. Our results show that the hypermethylation frequency of single genes and the accumulation of epigenetic alterations in individual samples of NSCLC patients may vary considerably. Molecular parameters such as hypermethylation of RASSF1A or p14(ARF) may be useful prognostic markers in subpopulations.

Gadgeel, S. M., S. Ali, et al. (2009). "Genistein enhances the effect of epidermal growth factor receptor tyrosine kinase inhibitors and inhibits nuclear factor kappa B in nonsmall cell lung cancer cell lines." Cancer **115**(10): 2165-76.

BACKGROUND: Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have shown modest clinical benefit in patients with relapsed nonsmall cell lung cancer (NSCLC). Down-regulation of Akt appears to correlate with the antitumor activity of EGFR-TKIs. Akt activates nuclear factor kappa B (NF-kappaB), which transcribes genes important for cell survival, invasion, and metastasis. The authors hypothesized that genistein, through the inhibition of NF-kappaB, could enhance the activity of EGFR-TKIs in NSCLCs. METHODS: Three NSCLC cell lines with various EGFR mutation status and sensitivities to EGFR-TKIs were selected: H3255 (L858R), H1650 (del E746-A750), and H1781 (wild-type EGFR). Cells were treated with erlotinib, gefitinib, genistein, or the combination of each of the EGFR-TKIs with genistein. Cell survival and apoptosis were assessed, and expression levels of EGFR, pAkt, cyclooxygenase-2 (COX-2), E-cadherin, prostaglandin E(2) (PGE(2)), and NF-kappaB were measured. RESULTS: Both EGFR-TKIs demonstrated growth inhibition and apoptosis in each of the cell lines, but H1650 and H1781 were much less sensitive. Genistein demonstrated some antitumor activity in all cell lines, but enhanced growth inhibition and apoptosis when combined with the EGFR-TKIs in each of the cell lines. Both combinations down-regulated NF-kappaB significantly more than either agent alone in H3255. In addition, the combinations reduced the expression of EGFR, pAkt, COX-2, and PGE(2,) consistent with inactivation of NF-kappaB. CONCLUSIONS: The authors concluded that genistein enhances the antitumor effects of EGFR-TKIs in 3 separate NSCLC cell lines. This enhanced activity is in part because of greater reduction in the DNA-binding activity of NF-kappaB when EGFR-TKIs were combined with genistein.

Gallegos Ruiz, M. I., K. Floor, et al. (2007). "EGFR and K-ras mutation analysis in non-small cell lung cancer: comparison of paraffin embedded versus frozen specimens." Cell Oncol **29**(3): 257-64.

BACKGROUND: Mutational analysis of the Epidermal Growth Factor Receptor (EGFR) and K-ras genes to select non-small cell lung cancer (NSCLC) patients for treatment with novel EGFR tyrosine kinase inhibitors is an appealing possibility currently under investigation. Although frozen tumor tissue would probably be the optimal source for analysis, the most common source of tumor material is fixed and paraffin embedded (FPE) archival specimens. Here, we evaluate how different procedures of tissue sample processing and preservation may affect the outcome of EGFR and K-ras mutation analysis. Furthermore, we compare the sensitivity of the analysis using genomic DNA (gDNA) versus RNA. METHODS: We used PCR amplification and direct sequencing to analyze EGFR and K-ras genes in paired FPE and frozen tumor samples corresponding to 47 NSCLC patients. In frozen samples, the analysis was carried out using both gDNA and RNA extracted in parallel. RESULTS: Whereas 100% of frozen samples were successfully amplified, the rate of successful PCR amplification in FPE samples was approximately 50%. We detected three previously described EGFR point mutations in 2 samples. In ten other samples, a K-ras mutation was observed. These mutations were detected in DNA extracted from frozen samples as well as in DNA obtained from FPE tissue. In addition, 10 nucleotide changes, were detected in FPE samples that were not detected in the frozen specimens. Upon re-analysis, these nucleotide changes could not be confirmed and were most likely the result of paraffin embedding and fixation procedures. All mutations found in gDNA were also detected in the corresponding RNA and, in two cases, the presence of the mutant allele was easier to identify by using RNA. CONCLUSIONS: Our results indicate that RNA extracted from frozen tissue is the preferred source for EGFR and K-ras mutation testing. When analyzing FPE samples, reducing the size of the amplified fragments would increase PCR success rate, and care should be taken to control for false-positive results.

Gao, Z., Z. Xu, et al. (2009). "Promoter demethylation of WIF-1 by epigallocatechin-3-gallate in lung cancer cells." Anticancer Res **29**(6): 2025-30.

BACKGROUND: Aberrant promoter methylation of Wnt inhibitory factor-1 (WIF-1) is a fundamental mechanism of epigenetic silencing in human cancers. Epigallocatechin-3-gallate (EGCG) has been reported to directly reactivate several methylation-silenced genes. The promoter demethylation and reactivation of WIF-1 has not

previously been reported. MATERIALS AND METHODS: Methylation-specific PCR, sequencing analysis and RT-PCR analysis were performed to evaluate promoter demethylation of WIF-1 and WIF-1 expression, Western blot analysis and luciferase reporter assay were performed to evaluate expression of cytosolic beta-catenin protein and Tcf/Lef reporter activity. RESULTS: Promoter demethylation of WIF-1 and restoration of WIF-1 expression after EGCG treatment are demonstrated in H460 and A549 cell lines. EGCG also decreased cytosolic beta-catenin protein level and inhibited Tcf/Lef reporter activity. CONCLUSION: These results suggest the potential therapeutic use of EGCG for the reversal of WIF-1 promoter methylation.

Gao, Z., Z. Xu, et al. (2009). "Procaine and procainamide inhibit the Wnt canonical pathway by promoter demethylation of WIF-1 in lung cancer cells." Oncol Rep **22**(6): 1479-84.

Secreted Wingless type (Wnt) ligands have previously been shown to be involved in tumor developmental processes and oncogenesis. Aberrant promoter methylation of Wnt inhibitory factor-1 (WIF-1) is a fundamental mechanism of epigenetic silencing in human cancers. Procaine, a local anesthetic drug, and procainamide, a drug for the treatment of cardiac arrhythmias, have been reported as inhibitors of DNA methylation, causing demethylation and reactivation of methylation-silenced genes such as RARbeta and GSTP1. The promoter demethylation of WIF-1 has not previously been reported on. We demonstrated previously that WIF-1 is silenced due to promoter hypermethylation in lung cancer cell lines. In this study, we demonstrate promoter demethylation of WIF-1; restoration of WIF-1 expression, and underexpression of cytosolic beta-catenin protein and TCF reporter activity, after procaine and procainamide treatment in H460 and A549 cell lines. Our results provide the first evidence that procaine and procainamide reactivate WIF-1 in these cancer cells and downregulate the Wnt canonical pathway. These results further suggest that procaine and procainamide may have a potential use for preventing the development of lung cancer.

Giaccone, G., M. Gallegos Ruiz, et al. (2006). "Erlotinib for frontline treatment of advanced non-small cell lung cancer: a phase II study." Clin Cancer Res **12**(20 Pt 1): 6049-55.

PURPOSE: Erlotinib has proven activity in pretreated patients with advanced non-small cell lung cancer (NSCLC). We evaluated erlotinib in the frontline treatment of advanced NSCLC and assessed biological predictors of outcome. EXPERIMENTAL DESIGN: In this phase II study, chemotherapy-naive patients with stage IIIB/IV NSCLC received oral erlotinib (150 mg/d) until disease progression or unacceptable toxicity occurred. Tumor response was assessed every 6 weeks, and samples were analyzed for potential molecular markers of treatment response and survival. The primary end point was the proportion of patients without disease progression after 6 weeks of treatment. RESULTS: Fifty-three patients were eligible. The overall rate of nonprogression at 6 weeks was 52.8% (28 of 53 patients). Tumor response rate was 22.7%, with 1 complete response, 11 partial responses, and 16 cases of stable disease. Responses were seen across most patient clinical characteristics. The median duration of tumor response was 333 days; median overall survival was 391 days; and median time to disease progression was 84 days. Erlotinib was well tolerated, the main treatment-related adverse events being mild-to-moderate rash and diarrhea. Histologic material for biological studies was available in 29 cases. Four of five responders and one patient with stable disease had a classic epidermal growth factor receptor tyrosine kinase mutation. Two progressing patients exhibited epidermal growth factor receptor point mutations (one with T790M mutation), and K-ras mutations were detected in 10 nonresponders. CONCLUSIONS: Erlotinib shows significant antitumor activity in the first-line treatment of advanced NSCLC and may be a viable alternative to chemotherapy. Patient selection cannot easily be based on clinical or biological variables.

Giaccone, G. and J. A. Rodriguez (2005). "EGFR inhibitors: what have we learned from the treatment of lung cancer?" Nat Clin Pract Oncol **2**(11): 554-61.

Tyrosine kinase inhibitors directed against the epidermal growth factor receptor (EGFR) are the first molecular-targeted agents to be approved in the US and other countries for the treatment of advanced non-small-cell lung cancer after failure of chemotherapy. Some patient characteristics, such as never-smoking, female gender, East Asian origin, adenocarcinoma histology, and bronchioloalveolar subtype, are associated with a greater benefit from treatment with EGFR inhibitors. Recently, studies have identified gene mutations targeting the kinase domain of the EGFR that are related to the response to inhibitors. Most EGFR mutations predict a higher benefit from treatment compared with wild-type receptors and are correlated with clinical features related to better outcome; some EGFR mutations, however, confer drug resistance. The analysis of material usually available from lung cancer patients, using techniques such as direct sequencing to determine EGFR mutational status, can be technically challenging. In this regard, high EGFR copy number

and EGFR protein detected by immunohistochemistry can also be used to select those patients who would benefit from treatment. Prospective validation of biological and clinical markers of sensitivity needs to be performed.

Gonlugur, U., H. Pinarbasi, et al. (2006). "The association between polymorphisms in glutathione S-transferase (GSTM1 and GSTT1) and lung cancer outcome." Cancer Invest **24**(5): 497-501.

BACKGROUND: Polymorphisms in the glutathione S-transferase (GST) family may be associated with increased risk of lung cancer, somatic changes in lung tumour tissue, and survival. We evaluated survival according to GST polymorphism in lung cancer patients. METHODS: We studied DNA polymorphisms of 81 primary lung cancer patients at 2 glutathione-related loci: GSTM1, and GSTT1 that encode glutathione S-transferase-mu, and glutathione S-transferase- square. The presences of the GSTM1 and GSTT1 genes were assayed by PCR. Kaplan-Meier with log rank tests, and Cox regression models were applied in the analysis. RESULTS: The median age of 75 males and 6 females was 60 years. Median survival of the whole population was 8 months. In the first presentation, none of the patients with GSTT1 null genotype but 30 percent of the patients with GSTT1-positive genotype had liver metastasis ($p < 0.01$) but GSTT1 genotype was not associated with survival. Sputum ($p < 0.01$) was more common in patients with GSTM1 null genotype. Subjects with the GSTM1-null genotype had shorter survival. Using a Cox proportional hazard model, GSTM1, tumor (T) factor and thoracic irradiation status were identified as independent prognostic factors. CONCLUSIONS: Our preliminary results showed that GSTM1-null genotype was associated with shorter survival.

Gopalan, B., I. Ito, et al. (2004). "Nanoparticle based systemic gene therapy for lung cancer: molecular mechanisms and strategies to suppress nanoparticle-mediated inflammatory response." Technol Cancer Res Treat **3**(6): 647-57.

Cancer gene therapy for the treatment of lung cancer has shown promise in the laboratory and in Phase I/II clinical trials. However, it is currently limited to treating localized tumors due to host-immunity against the gene delivery vector and the transgene. Therefore, there is a tremendous effort to develop and test alternate gene delivery vectors that are efficient, non-immunogenic, and applicable for systemic therapy. One such gene delivery vehicle is the non-viral vector, DOTAP:cholesterol (DOTAP:Chol) nanoparticle. Preclinical studies from our laboratory has shown that DOTAP:Chol. nanoparticles are effective systemic gene delivery vectors that efficiently deliver tumor-suppressor genes to disseminated lung tumors. Based on our findings we have recently initiated a Phase-I trial for systemic treatment of lung cancer using a novel tumor suppressor gene, FUS1. Although DOTAP:Chol. nanoparticles complexed to DNA (DNA-nanoparticles) are efficient vectors for systemic therapy, induction of an inflammatory response in a dose-dependent fashion has also been observed thereby limiting its use. A better understanding of the underlying mechanism for DNA-nanoparticles-mediated inflammatory response will allow us to develop strategies to suppress inflammation and expand the therapeutic window in treating human cancer. In the present study we conducted experiments examining the mechanism of nanoparticle-mediated inflammatory response in vitro and in vivo. We demonstrate that systemic administration of DNA-nanoparticles induced multiple signaling molecules both in vitro and in vivo that are associated with inflammation. Use of small molecule inhibitors against the signaling molecules resulted in their suppression and thereby reduced inflammation without affecting transgene expression. Our results provide a rationale to use small molecule inhibitors to suppress nanoparticle-mediated inflammation when administered systemically. Further development and testing will allow us to incorporate this strategy into future clinical trials that is based on systemic non-viral vector gene therapy.

Han, S. W., T. Y. Kim, et al. (2006). "Optimization of patient selection for gefitinib in non-small cell lung cancer by combined analysis of epidermal growth factor receptor mutation, K-ras mutation, and Akt phosphorylation." Clin Cancer Res **12**(8): 2538-44.

PURPOSE: Mutations in epidermal growth factor receptor (EGFR) are strongly predictive of gefitinib efficacy in non-small-cell lung cancer. However, the presence of EGFR mutant nonresponses and nonmutant responses points out the need for more comprehensive analysis. Patients and Methods: For 69 non-small-cell lung cancer patients treated with gefitinib, we have extended our analysis to EGFR gene copy number by fluorescence in situ hybridization, mutations in K-ras, HER2, and exon 20 of EGFR by direct sequencing, and phosphatase and tensin homologue expression by immunohistochemistry, in addition to EGFR exons 18, 19, and 21, and phosphorylations of Akt and extracellular signal-regulated kinase reported previously. RESULTS: EGFR mutation and high gene copy number were associated with better objective response in univariate analysis. However, only gefitinib-sensitive EGFR mutation was independently predictive of both response ($P = 0.011$) and survival (P

= 0.002) in multivariate analysis. No patients with K-ras mutation, including two EGFR mutants, showed response. In EGFR nonmutants, patients with either K-ras mutation or p-Akt overexpression exhibited poor response and time-to-progression whereas patients with high gene copy number tended to have better outcomes in univariate analysis. In multivariate analysis of time-to-progression in EGFR nonmutants, K-ras mutation or p-Akt overexpression was associated with shorter time-to-progression (P = 0.017). No patient with HER2 mutation showed response to gefitinib. Reduced phosphatase and tensin homologue expression was not associated with gefitinib sensitivity. CONCLUSION: Gefitinib-sensitive EGFR mutation is the single most important predictor of gefitinib sensitivity. In addition to EGFR mutation, K-ras mutation and Akt phosphorylation aid in better prediction of gefitinib responsiveness in non-small-cell lung cancer.

He, B., L. You, et al. (2003). "SOCS-3 is frequently silenced by hypermethylation and suppresses cell growth in human lung cancer." Proc Natl Acad Sci U S A **100**(24): 14133-8.

Lung cancer is the leading cause of cancer death in the world, but the molecular mechanisms for its development have not been well characterized. The suppressors of cytokine signaling (SOCS) are inhibitors of cytokine signaling that function via the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway. Eight SOCS proteins with similar structures have been identified so far. SOCS family members, however, have distinct mechanisms of inhibition of JAK/STAT signaling. Abnormalities of the JAK/STAT pathway are associated with cancer. Inhibition of signaling results in growth suppression in various cell types. Recently, the involvement of SOCS-1 in carcinogenesis has been reported. Here, we report identification of frequent hypermethylation in CpG islands of the functional SOCS-3 promoter that correlates with its transcription silencing in cell lines (lung cancer, breast cancer, and mesothelioma) and primary lung cancer tissue samples. Restoration of SOCS-3 in lung cancer cells where SOCS-3 was methylation-silenced resulted in the down-regulation of active STAT3, induction of apoptosis, and growth suppression. Our results suggest that methylation silencing of SOCS-3 is one of the important mechanisms of constitutive activation of the JAK/STAT pathway in cancer pathogenesis. The data also suggest that SOCS-3 therapy may be useful in the treatment of cancer.

He, B., L. You, et al. (2004). "Activity of the suppressor of cytokine signaling-3 promoter in human non-small-cell lung cancer." Clin Lung Cancer **5**(6): 366-70.

The Janus kinase (JAKs)/signal transducers and activators of transcription (STAT) signaling pathway is controlled by a classical feedback loop through suppressors of cytokine signaling (SOCS/JAB/SSI). Suppressors of cytokine signaling proteins are induced rapidly by activated STATs upon phosphorylation and act to block the cytokine signal. Abnormalities of the JAK/STAT pathway are associated with cancer. Recently, we cloned the functional 5' promoter region of the human SOCS-3 gene and showed that this region is highly conserved in murine and rat SOCS-3 promoters. In addition, we found that the wild type SOCS-3 promoter construct has significantly greater activity in human non-small-cell lung cancer (NSCLC) cell lines than in normal cells in accordance with STAT3 deregulation in these cells. Furthermore, we have confirmed that frequent hypermethylation of the functional SOCS-3 promoter correlates with its transcription silencing in NSCLC cell lines and primary lung cancer tissue samples. Restoration of SOCS-3 in lung cancer cells in which SOCS-3 has been methylation-silenced induces apoptosis and suppresses growth. Therefore, methylation silencing of SOCS-3 may be used as a marker for early detection of NSCLC. Suppressor of cytokine signaling-3 therapy may be useful for the treatment of lung cancer.

Helfrich, B. A., D. Raben, et al. (2006). "Antitumor activity of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib (ZD1839, Iressa) in non-small cell lung cancer cell lines correlates with gene copy number and EGFR mutations but not EGFR protein levels." Clin Cancer Res **12**(23): 7117-25.

PURPOSE: Recognition that the epidermal growth factor receptor (EGFR) was a therapeutic target in non-small cell lung cancer (NSCLC) and other cancers led to development of the small-molecule receptor tyrosine kinase inhibitors gefitinib and erlotinib. Clinical trials established that EGFR tyrosine kinase inhibitors produced objective responses in a minority of NSCLC patients. We examined the sensitivity of 23 NSCLC lines with wild-type or mutated EGFR to gefitinib to determine genes/proteins related to sensitivity, including EGFR and HER2 cell surface expression, phosphorylated EGFR expression, EGFR gene copy number, and EGFR mutational status. Downstream cell cycle and signaling events were compared with growth-inhibitory effects. EXPERIMENTAL DESIGN: We determined gefitinib sensitivity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays, EGFR expression by fluorescence-

activated cell sorting and immunohistochemistry, phosphorylated EGFR by Western blotting, EGFR gene copy number by fluorescence in situ hybridization, and EGFR mutation by sequencing. The cellular effects of gefitinib on cell cycle were determined by flow cytometry and the molecular effects of gefitinib EGFR inhibition on downstream signal proteins by Western blotting. Gefitinib in vivo effects were evaluated in athymic nude mice bearing sensitive and resistant NSCLC xenografts. RESULTS: There was a significant correlation between EGFR gene copy number, EGFR gene mutations, and gefitinib sensitivity. EGFR protein was necessary but not sufficient for predicting sensitivity. Gefitinib-sensitive lines showed a G(1) cell cycle arrest and inactivation of downstream signaling proteins; resistant cell lines had no changes. The in vivo effects mirrored the in vitro effects. CONCLUSIONS: This panel of NSCLC lines characterized for gefitinib response was used to identify predictive molecular markers of response to gefitinib. Several of these have subsequently been shown to identify NSCLC patients likely to benefit from gefitinib therapy.

Homma, S., Y. Ishii, et al. (2009). "Nrf2 enhances cell proliferation and resistance to anticancer drugs in human lung cancer." Clin Cancer Res **15**(10): 3423-32.

PURPOSE: NF-E2-related factor 2 (Nrf2), a key transcription regulator for antioxidant and detoxification enzymes, is abundantly expressed in cancer cells. In this study, therefore, the role of Nrf2 in cancer cell proliferation and resistance to anticancer drugs was investigated. EXPERIMENTAL DESIGN: We used three human lung cancer cell lines with different degrees of Nrf2 activation: Nrf2 was highly activated in A549 cells, slightly activated in NCI-H292 cells, and not activated in LC-AI cells under unstimulated conditions. Result: A549 cells showed higher resistance to cisplatin compared with NCI-H292 and LC-AI cells. The resistance to cisplatin was significantly inhibited in A549 but not in NCI-H292 or LC-AI cells by knockdown of Nrf2 with its specific small interfering RNA (Nrf2-siRNA). The cell proliferation was also most prominently inhibited in A549 cells by treatment with Nrf2-siRNA. In A549 cells, the expression of self-defense genes, such as antioxidant enzymes, phase II detoxifying enzymes, and drug efflux pumps, was significantly reduced by Nrf2-siRNA concomitant with a reduction of the cellular glutathione level. The degree of DNA crosslink and apoptosis after treatment with cisplatin was significantly elevated in A549 cells by Nrf2-siRNA. Knockdown of Nrf2 arrested the cell cycle at G(1) phase with a reduction of the phosphorylated form of retinoblastoma protein in A549 and NCI-H292 cells but not in LC-AI cells. CONCLUSION: These results indicate that the Nrf2 system is essential for both cancer cell proliferation and resistance to anticancer drugs. Thus, Nrf2 might be a potential target to enhance the effect of anticancer drugs.

Hotta, K., K. Kiura, et al. (2007). "Clinical significance of epidermal growth factor receptor gene mutations on treatment outcome after first-line cytotoxic chemotherapy in Japanese patients with non-small cell lung cancer." J Thorac Oncol **2**(7): 632-7.

INTRODUCTION: The relationship between the EGFR gene mutation status and clinical outcome has not fully been assessed in patients with non-small cell lung cancer (NSCLC) who received cytotoxic agents. The aim of this study was to clarify its association. We also examined whether this association could be affected by previous gefitinib treatment. METHODS: Patients with advanced or postoperative recurrent NSCLC who received both cytotoxic chemotherapy and gefitinib monotherapy in their treatment course were included in this study. An EGFR mutation was determined in exons 19 and 21 by direct sequencing. RESULTS: Of 194 Japanese patients with advanced or relapsed NSCLC assessable for mutation analysis, 60 received both cytotoxic chemotherapy and gefitinib monotherapy through their treatment courses. EGFR mutations significantly affected progression-free survival (PFS) in the first-line cytotoxic chemotherapy regimens in the multivariate analysis (hazard ratio for PFS = 0.422; p = 0.0422). In contrast, in 28 (47%) of the 60 patients who also received cytotoxic chemotherapy after the relapse to gefitinib monotherapy, there were no differences in PFS stratified by EGFR mutation status. The sensitivity to gefitinib was, however, correlated with EGFR mutation status, and its sensitivity was retained even in the second-line treatment setting in patients with EGFR mutations. CONCLUSIONS: EGFR mutations were therefore significantly associated with a better PFS in the first-line cytotoxic chemotherapy regimens. However, its association was not observed in the cytotoxic regimens administered after the relapse to gefitinib monotherapy, whereas gefitinib sensitivity was associated with an EGFR mutation even in the second-line or later treatment settings.

Hsieh, M. H., Y. F. Fang, et al. (2006). "Complex mutation patterns of epidermal growth factor receptor gene associated with variable responses to gefitinib treatment in patients with non-small cell lung cancer." Lung Cancer **53**(3): 311-22.

Mutational analysis was performed in the kinase domain (exons 18-21) of the EGFR gene on tumor tissues of 65 non-small cell lung cancer

(NSCLC) patients who had received gefitinib monotherapy. The association between EGFR gene mutation, gefitinib treatment response, and the overall survival were evaluated. In total, EGFR mutations with complex patterns were identified in 32 tumors. The overall mutation rate was 49.2% (32/65). Twenty of the 32 patients were responders, 10 non-responders, and 2 not assessable. The most common mutation in non-responders was L858R. Gefitinib responsiveness was only significantly associated with EGFR mutation and adenocarcinoma. The median survival for responder (15.5 months) was much longer than non-responder (9.23 months), though the difference only had marginal significance (p=0.056). The difference of overall survival between patients with and without EGFR mutation was non-significant (p=0.7819), mainly due to the short survival of the non-responders with EGFR mutations (median survival=6.2 months). Our study revealed that the response to gefitinib treatment in NSCLC patients with EGFR mutations could be quite variable even for the same EGFR mutation type. An analysis of the various EGFR mutations and the response patterns was also performed and compared with recently published reports on EGFR mutation and gefitinib responsiveness.

Hsu, D. S., C. R. Acharya, et al. (2009). "Characterizing the developmental pathways TTF-1, NKX2-8, and PAX9 in lung cancer." Proc Natl Acad Sci U S A **106**(13): 5312-7.

We investigated the clinical implications of lung developmental transcription factors (TTF-1, NKX2-8, and PAX9) that we recently discovered as cooperating oncogenes activated by way of gene amplification at chromosome 14q13 in lung cancer. Using stable transfectants of human bronchial epithelial cells, RNA expression profiles (signatures) representing activation of the biological pathways defined by each of the 3 genes were determined and used to risk stratify a non-small-cell lung cancer (NSCLC) clinical data set consisting of 91 early stage tumors. Coactivation of the TTF-1 and NKX2-8 pathways identified a cluster of patients with poor survival, representing approximately 20% of patients with early stage NSCLC, whereas activation of individual pathways did not reveal significant prognostic power. Importantly, the poor prognosis associated with coactivation of TTF-1 and NKX2-8 was validated in 2 other independent clinical data sets. Furthermore, lung cancer cell lines showing coactivation of the TTF-1 and NKX2-8 pathways were shown to exhibit resistance to cisplatin, the standard of care for the treatment of NSCLC. This suggests that the cohort of patients with coactivation of TTF-1 and NKX2-8 pathways appears to be resistant to standard cisplatin therapy, suggesting the need for alternative therapies in this cohort of high-risk patients.

Hsu, H. S., C. K. Wen, et al. (2005). "Promoter hypermethylation is the predominant mechanism in hMLH1 and hMSH2 deregulation and is a poor prognostic factor in nonsmoking lung cancer." Clin Cancer Res **11**(15): 5410-6.

PURPOSE AND EXPERIMENTAL DESIGN: The etiologic association and prognostic significance of mismatch repair gene/protein alterations have never been examined in nonsmoking lung cancer. Therefore, we investigated protein expression and promoter hypermethylation of hMLH1 and hMSH2 genes in the tumor specimens from 105 nonsmoking female non-small cell lung cancer (NSCLC) patients. Immunohistochemistry and restriction enzyme-based multiplex PCR were used to examine the protein expression and promoter hypermethylation, respectively. The occurrence of gene/protein alteration for each gene was compared with the patients' clinicopathologic variables as well as the overall survival and cancer-specific survival rates. RESULTS: Protein expression alteration and promoter hypermethylation were observed in 66% to 67% and 30% to 34% of tumor specimens for hMLH1 and hMSH2 genes, respectively. Loss of hMLH1 and hMSH2 protein expression was significantly associated with their promoter hypermethylation ($P < 0.0001$ and $P = 0.049$). The overall survival and cancer-specific survival rates were significantly lower in patients with promoter hypermethylation of hMSH2 gene than in those without hypermethylation ($P = 0.038$ and $P = 0.004$). The poor prognosis was still especially significant in adenocarcinoma ($P = 0.035$ and $P = 0.061$) and early-stage NSCLC patients ($P = 0.067$ and $P = 0.041$). CONCLUSION: Our data suggest that hMLH1 is the major altered mismatch repair gene involved in nonsmoking NSCLC tumorigenesis and that promoter methylation is the predominant mechanism in hMLH1 and hMSH2 deregulation. In addition, promoter methylation of the hMSH2 gene may be a potential prognostic factor in nonsmoking female lung cancer.

Ikuta, K., K. Takemura, et al. (2005). "Overexpression of constitutive signal transducer and activator of transcription 3 mRNA in cisplatin-resistant human non-small cell lung cancer cells." Oncol Rep **13**(2): 217-22.

Non-small cell lung cancer (NSCLC) often shows intrinsic multidrug resistance, which is one of the most serious problems in cisplatin-based adjuvant chemotherapy. Recently, the constitutive activation of signal transducer and activator of transcription

(STAT) factors has been found in a variety of human cancers. In the present study, the mRNA expression of STATs in various human NSCLC cell lines was investigated by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) to determine whether STATs can be implicated in cisplatin resistance and apoptosis inducibility. Cisplatin triggered apoptosis in Ma-46 based on biochemical and morphological findings, but not in Ma-31. The mRNA expression of STAT3 was highest in cisplatin-resistant Ma-31 and lowest in cisplatin-sensitive Ma-46. A 6-hour exposure of cancer cells to cisplatin failed to stimulate STAT3 mRNA expression. Therefore, an increased transcriptional level of constitutive STAT3 may be related to the suppressive regulation of the apoptotic pathway in intrinsically chemo-resistant NSCLC cells.

Inoue, A. and T. Nukiwa (2005). "Gene mutations in lung cancer: promising predictive factors for the success of molecular therapy." PLoS Med **2**(1): e13.

Inoue, A., T. Suzuki, et al. (2006). "Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations." J Clin Oncol **24**(21): 3340-6.

PURPOSE: This study was undertaken to investigate the efficacy and the feasibility of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutations. PATIENTS AND METHODS: The EGFR gene status in various tumor samples obtained from chemotherapy-naive advanced NSCLC patients was examined by DNA sequencing of EGFR exons 18 to 23. Patients harboring EGFR mutations received gefitinib (250 mg/d) alone. The response rate, progression-free survival (PFS), and toxicity profile were assessed prospectively. RESULTS: Between June 2004 and October 2005, 75 patients were examined for the EGFR status, and 25 patients (33%) harbored EGFR mutations. EGFR mutations were significantly frequent in females ($P < .01$) and never or light smokers ($P < .001$). Sixteen patients with EGFR mutations were enrolled onto the study. The overall response rate in these patients was 75% (95% CI, 54% to 96%), and the disease control rate was 88% (95% CI, 71% to 100%). The median PFS time of these patients was 9.7 months (95% CI, 7.4 to 9.9 months). No life-threatening toxicity was observed. CONCLUSION: Treatment with gefitinib alone for chemotherapy-naive NSCLC patients with EGFR mutations could achieve a high efficacy with acceptable toxicity. To assess the proper timing of gefitinib in such patients, a subsequent randomized trial comparing gefitinib with standard chemotherapy is warranted.

Jackman, D. M., B. Y. Yeap, et al. (2006). "Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib." Clin Cancer Res **12**(13): 3908-14.

PURPOSE: Somatic mutations in the epidermal growth factor receptor (EGFR) have been detected in patients with non-small cell lung cancer (NSCLC) and are associated with sensitivity to treatment with gefitinib or erlotinib. Our study explored the relationship between the two most common types of somatic EGFR mutations, exon 19 deletions and the L858R point mutation, and outcomes of patients following treatment with gefitinib or erlotinib. EXPERIMENTAL DESIGN: Tumor specimens obtained before treatment with gefitinib or erlotinib were analyzed for EGFR mutations. Patients with exon 19 deletion or L858R mutations were identified. The response rate, time to progression, and overall survival were determined for the two groups. RESULTS: We identified 36 patients with NSCLC and an EGFR mutation who were treated with gefitinib or erlotinib. Patients with an exon 19 deletion had a significantly longer overall survival compared with patients with an L858R mutation (38 versus 17 months; $P = 0.04$). There were also trends toward higher response rate (73% versus 50%) and improved time to progression (24 versus 10 months) for the patients with an exon 19 deletion, although these were not independently significant in a multivariate analysis. A difference in response rate for patients treated with gefitinib compared with erlotinib was also noted [18 of 23 (78%) versus 3 of 9 (33%); $P = 0.04$]. No obvious difference in time to progression or overall survival was noted between gefitinib- and erlotinib-treated patients. CONCLUSIONS: Patients with NSCLC and EGFR exon 19 deletions have a longer survival following treatment with gefitinib or erlotinib compared with those with the L858R mutation. Pooling of greater numbers of patients and completion of prospective trials are needed to further define the predictive and prognostic roles of different EGFR mutations with respect to treatment with gefitinib, erlotinib, and other EGFR inhibitors.

Jeon, Y. K., S. W. Sung, et al. (2006). "Clinicopathologic features and prognostic implications of epidermal growth factor receptor (EGFR) gene copy number and protein expression in non-small cell lung cancer." Lung Cancer **54**(3): 387-98.

Increased epidermal growth factor receptor (EGFR) gene copy numbers and mutations predict

sensitivity to EGFR tyrosine kinase inhibitor in non-small cell lung cancer (NSCLC). However, the clinicopathologic features of EGFR gene copy status in NSCLC remain unclear. We retrospectively analyzed 262 cases of NSCLC, including 135 squamous cell carcinomas (SCC) and 112 adenocarcinomas (ADC), for which paraffin blocks of the resected primary lung mass were available. None had received EGFR-targeted therapy. EGFR gene copy number was evaluated using fluorescence in situ hybridization (FISH), and EGFR expression was determined immunohistochemically using a tissue microarray. A high EGFR gene copy (EGFR FISH-positive) was found in 30.2% of the cases (amplification in 11.1% and high polysomy in 19.1%). There was no significant difference in EGFR FISH status with respect to SCC and ADC histology. The EGFR FISH-positive rate was higher in non-smokers than in smokers in the multivariate analysis (p=0.012). EGFR expression was significantly associated with a high EGFR gene copy and SCC histology (p=0.000). In the univariate survival analysis, EGFR FISH-positivity predicted worse survival in SCC (p=0.059), especially stage I SCC (p=0.04). EGFR amplification was associated with a shorter survival in node-positive SCC (p=0.015). However, the EGFR gene copy number or protein expression had no influence on the prognosis of ADC. In conclusion, the EGFR FISH-positive rate in Korean patients with NSCLC was similar to rates in Western populations, unlike the higher frequencies of EGFR mutation in East Asians. A high EGFR gene copy number was significantly more common in non-smokers, as were EGFR mutations. A high EGFR gene copy number predicted worse survival in SCC; therefore, the prognostic implications of the EGFR gene and protein should be analyzed in the context of histology and staging in NSCLC.

John, T., G. Liu, et al. (2009). "Overview of molecular testing in non-small-cell lung cancer: mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors." Oncogene **28 Suppl 1**: S14-23.

Most patients with non-small-cell lung cancer (NSCLC) present with advanced disease. Current treatment paradigms are shifting from cytotoxic chemotherapies alone to single-agent and combination biological and targeted therapies. As patient responses to these therapies vary, predictive biomarkers will be an important facet of a patient's diagnostic workup in personalized medicine, as there is accumulating evidence that they may enable the prognostication and prediction of therapeutic response. Potential biomarkers for the selection of patients with NSCLC most likely to benefit from epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, include mutations, gene copy number increase and single-nucleotide polymorphisms of the EGFR gene, EGFR protein expression and oncogenic mutation on the KRAS gene. Many techniques are available to assay for these biomarkers. In this review, we present the current weight of evidence for using these methods as biomarkers for anti-EGFR therapy in patients with NSCLC.

Kang, Y., J. A. Hong, et al. (2007). "Dynamic transcriptional regulatory complexes including BORIS, CTCF and Sp1 modulate NY-ESO-1 expression in lung cancer cells." Oncogene **26**(30): 4394-403.

Previously, we reported that the paralogous zinc-finger proteins--CTCF and brother of the regulator of imprinted sites (BORIS), directly contribute to transcriptional regulation of NY-ESO-1 in lung cancer cells. To further examine mechanisms that mediate expression of this cancer-testis gene, we performed software-guided analysis of the NY-ESO-1 promoter region, which revealed several potential Sp1-binding motifs. Sequential 5-aza-2'deoxycytidine/depsipeptide FK228 treatment markedly induced BORIS expression and enhanced nuclear translocation of Sp1 in lung cancer cells. Transient transfection assays using promoter-reporter constructs, as well as gel-shift and chromatin immunoprecipitation experiments revealed that NY-ESO-1 promoter activity coincided with occupancy of the proximal Sp1-binding site in lung cancer cells. Mutations within the Sp1 recognition sequence specifically eliminated binding of Sp1 to this motif in vitro, and markedly diminished NY-ESO-1 promoter activity in vivo. siRNA-mediated inhibition of Sp1 expression decreased NY-ESO-1 promoter activity, whereas knock down of CTCF expression augmented NY-ESO-1 transcription in lung cancer cells. Co-immunoprecipitation experiments indicated that Sp1 physically interacts with BORIS but not with CTCF in vivo. Collectively, these findings suggest that BORIS recruits Sp1 to mediate de-repression of NY-ESO-1 during pulmonary carcinogenesis.

Kanteti, R., V. Nallasura, et al. (2009). "PAX5 is expressed in small-cell lung cancer and positively regulates c-Met transcription." Lab Invest **89**(3): 301-14.

PAX5 is a nuclear transcription factor required for B cell development, and its expression was evaluated in upper aerodigestive malignancies and pancreatic cancer by immunoblotting. The PAX5 protein expression was relatively strong in small-cell

lung cancer (SCLC, 11/12); however, its expression was not detected in non-SCLC (NSCLC, n=13), mesothelioma (n=7), pancreatic (n=6), esophageal (n=6) and head and neck cancer cell lines (n=12). In comparison, PAX8 and PAX3 expressions were absent or non-detectable in SCLC cell lines; however, PAX8 was expressed in most of the tested NSCLC cell lines (13/13) and also frequently in all the other cell lines. We also detected frequent expressions of PAX2 and PAX9 protein in the various cell lines. Utilizing neuroendocrine tumor samples, we found that the frequency as well as the average intensity of the expression of PAX5 increased from pulmonary carcinoid (9%, moderate and strong PAX5 expression, n=44), to large-cell neuroendocrine carcinoma (LCNC, 27%, n=11) to SCLC (33%, n=76). FISH analysis revealed no translocations of the PAX5 gene, but polyploidy in some SCLC tumor tissues (6/37). We determined that PAX5 could regulate the transcription of c-Met using luciferase-coupled reporter and chromatin immunoprecipitation analysis. In addition, the phospho-c-Met (active form) and PAX5 were both localized to the same intra-nuclear compartment in hepatocyte growth factor treated SCLC cells and interacted with each other. Finally, we determined the therapeutic translational potential of PAX5 using PAX5 knockdown SCLC cells in conjunction with Topoisomerase 1 (SN38) and c-Met (SU11274) inhibitors. Loss of endogenous PAX5 significantly decreased the viability of SCLC cells, especially when combined with SN38 or SU11274, and maximum effect was seen when both inhibitors were used. Therefore, we propose that PAX5 could be an important regulator of c-Met transcription and a potential target for therapy in SCLC.

Kanteti, R., S. Yala, et al. (2009). "MET, HGF, EGFR, and PXN gene copy number in lung cancer using DNA extracts from FFPE archival samples and prognostic significance." J Environ Pathol Toxicol Oncol **28**(2): 89-98.

Gene copy number analysis for some of the important molecules in lung tumorogenesis, such as MET, hepatocyte growth factor [(HGF), ligand for MET), epidermal growth factor receptor (EGFR), and paxillin (PXN), is likely to determine both the type of treatment and prognosis. Formalin-fixed paraffin-embedded (FFPE) archival tumor tissue samples are an excellent source for determining key molecular changes in the OncoGenome; however, existing extraction procedures yield relatively poor quality genomic DNA fragments. Although FISH is the method of choice for determining amplification of a gene, a more rapid quantitative poly-merase chain reaction (qPCR) technique to determine gene copy number can be used when reasonably good quality genomic DNA is available. We report here a relatively rapid method based on microwave/chelex-100 treatment that gives rise to genomic DNA fragments ranging from 1 to 12 Kb and beyond, thereby attesting to its superior quality. Genomic PCR for beta-globin gene gave reliable and reproducible results. The number of steps for extracting the DNA was kept to a minimum, and instead of precipitating the DNA, we preserved the genomic DNA extracts so as to prevent a loss in DNA yield. We found the extracts to be stable and amenable to qPCR and mutational analysis. Using lung adenocarcinoma FFPE samples and cell lines derived from lung adenocarcinomas, we demonstrated that the gene copy number for MET in lung adenocarcinoma tissue samples was preferentially increased over EGFR, HGF, and PXN and that it positively correlated with a better prognosis. In contrast, the genomic DNA extracted from 25 NSCLC cell lines gave a relatively higher gene copy number for all four genes evaluated. Our results indicate that the microwave/chelex-100-based method yields good-quality genomic DNA extracts that can be used for complex DNA analysis, such as determination of gene copy number. In addition, our data demonstrated that the adenocarcinoma cell lines potentially evolved under ex vivo conditions, and therefore, in genetic studies it is imperative to use primary tumors for generalized conclusions about lung tumors.

Kim, J. S., J. W. Kim, et al. (2006). "Cohypermethylation of p16 and FHIT promoters as a prognostic factor of recurrence in surgically resected stage I non-small cell lung cancer." Cancer Res **66**(8): 4049-54.

Despite advances in the detection and treatment of lung cancer, the prognosis for patients with lung cancer is poor, partly as a result of recurrences. We retrospectively analyzed the relationship between recurrence and survival in patients with non-small cell lung cancers (NSCLC), and the promoter methylation of p16, GSTP1, FHIT, H-cadherin, and RARbeta2 genes to identify a prognostic molecular marker associated with the recurrence of NSCLC. Methylation status from 335 paraffin blocks was determined by methylation-specific PCR. Of the 335 NSCLC samples, promoter methylation was detected in 35% for p16, 39% for RARbeta2, 42% for H-cadherin, 7% for GSTP1, and 21% for FHIT. Recurrence was observed in 39% (132 of 335) of the patients. Recurrence was significantly associated with histology (P = 0.001) and pathologic stage (P = 0.009). Hypermethylation of any single gene was not associated with recurrence in patients. However, cohypermethylation of p16 and FHIT genes in stage I NSCLCs was associated with an increased

risk of recurrence [odds ratio, 6.43; 95% confidence interval (CI), 1.04-20.19; P = 0.02] and poor recurrence-free survival after surgery (hazard ratio, 2.03; 95% CI, 1.09-6.23; P = 0.02). In addition, their survival after recurrence was also 4.62 times poorer (95% CI, 1.27-16.48; P = 0.005) than for those without cohypermethylation of both genes. In conclusion, the present study suggests that cohypermethylation of p16 and FHIT genes in patients with stage I NSCLC may be a valuable biomarker for predicting the recurrence-associated prognosis of the disease.

Kimura, H., K. Kasahara, et al. (2006). "EGFR mutation of tumor and serum in gefitinib-treated patients with chemotherapy-naive non-small cell lung cancer." J Thorac Oncol **1**(3): 260-7.

BACKGROUND: The authors evaluate the efficacy and safety of gefitinib monotherapy in chemotherapy-naive patients with advanced non-small-cell lung cancer (NSCLC). A secondary endpoint is to evaluate the relationship between clinical manifestations and epidermal growth factor receptor (EGFR) mutation status. METHODS: Japanese chemotherapy-naive NSCLC patients were enrolled. They had measurable lesions, Eastern Cooperative Oncology Group performance status of 0 to 2, and adequate organ and bone marrow function. Patients received 250 mg of oral gefitinib daily. EGFR mutations in exon 18, 19, and 21 of DNA extracted from tumor and serum were analyzed by genomic polymerase chain reaction and direct sequence. RESULTS: All 30 patients were eligible for the assessment of efficacy and safety. An objective response and stable disease were observed in 10 patients (33.3%) and nine patients (30.0%), respectively. The median time to progression was 3.3 months and the median overall survival was 10.6 months. The 1-year survival rate was 43.3%. Grade 3 toxicities were observed in seven patients. EGFR mutation was observed in four of 13 (30.8%) tumors, and two of them achieved partial response. In serum samples, three of 10 patients with EGFR mutations in the serum before treatment had a response to gefitinib. EGFR mutation was observed in 10 of 27 and significantly more frequently observed in the posttreatment samples from patients with a partial response or stable disease than in those from patients with progressive disease (p = 0.006). CONCLUSIONS: Gefitinib monotherapy in chemotherapy-naive NSCLC patients was active, with acceptable toxicities. These results warrant further evaluation of gefitinib monotherapy as a first-line therapy. The EGFR mutation in serum DNA may be a biomarker for monitoring the response to gefitinib during treatment.

Kluge, A., S. Dabir, et al. (2009). "Cooperative interaction between protein inhibitor of activated signal transducer and activator of transcription-3 with epidermal growth factor receptor blockade in lung cancer." Int J Cancer **125**(7): 1728-34.

Epidermal Growth Factor Receptor (EGFR) targeting in nonsmall cell lung cancer (NSCLC) is an established treatment modality; however, it only benefits a minority of patients. STAT3 (signal transducer and activator of transcription-3) plays an important role in the oncogenic signal transduction pathway of NSCLC. Inhibition of STAT3 results in NSCLC growth inhibition and apoptosis. We have previously shown that combined inhibition of EGFR and STAT3 by small molecules resulted in improved therapeutic efficacy as compared with blocking EGFR alone. However, the STAT3 protein has a number of endogenous negative regulators including PIAS3 (Protein Inhibitor of Activated STAT3). In this study, we investigated for the first time the role of PIAS3 in modulating oncogenic EGFR-STAT3 signaling pathway in lung cancer and the anti-proliferative effect of using PIAS3 in conjunction with EGFR blockade in NSCLC. We demonstrate that PIAS3 is expressed in variable degrees in all NSCLC cells. EGF and IL-6 stimulation resulted in the association of PIAS3 with STAT3. The PIAS3/STAT3 complex then bound the STAT3 DNA binding sequence resulting in STAT3 regulated gene expression. Over-expression of PIAS3, using a PIAS3 expression construct, decreases STAT3 transcriptional activity. Furthermore, over-expression of PIAS3 consistently decreased proliferation. EGFR blockade and PIAS3 over-expression in combination had significantly greater anti-proliferative effects as compared with either EGFR blockade or PIAS3 over-expression alone. In conclusion, PIAS3 is expressed in NSCLC cell lines and its over-expression decreased STAT3 transcriptional activity, decreased proliferation of NSCLC cells and when used in conjunction with EGFR inhibitors, increased the anti-proliferative effects.

Kobayashi, S., T. J. Boggon, et al. (2005). "EGFR mutation and resistance of non-small-cell lung cancer to gefitinib." N Engl J Med **352**(8): 786-92.

Mutations of the epidermal growth factor receptor (EGFR) gene have been identified in specimens from patients with non-small-cell lung cancer who have a response to anilinoquinazoline EGFR inhibitors. Despite the dramatic responses to such inhibitors, most patients ultimately have a relapse. The mechanism of the drug resistance is unknown. Here we report the case of a patient with EGFR-mutant, gefitinib-responsive, advanced non-

small-cell lung cancer who had a relapse after two years of complete remission during treatment with gefitinib. The DNA sequence of the EGFR gene in his tumor biopsy specimen at relapse revealed the presence of a second point mutation, resulting in threonine-to-methionine amino acid change at position 790 of EGFR. Structural modeling and biochemical studies showed that this second mutation led to gefitinib resistance.

Kondo, M., T. Yokoyama, et al. (2005). "Mutations of epidermal growth factor receptor of non-small cell lung cancer were associated with sensitivity to gefitinib in recurrence after surgery." Lung Cancer **50**(3): 385-91.

The epidermal growth factor receptor (EGFR) gene has recently been reported to be mutated in a subset of non-small cell lung cancers (NSCLC), with the mutations being correlated with the patients' drug sensitivity to gefitinib, an EGFR kinase inhibitor. In this study, we searched for EGFR mutations in patients with lung cancer using primary tumor specimens obtained at initial surgery and examined whether their recurrent tumors showed a response to gefitinib depending on the presence of the activating mutation. Among 12 lung cancers that were treated with gefitinib after recurrence, we found that all four tumors which showed a response to gefitinib had an activating mutation in EGFR, whereas none of the remaining eight tumors had a mutation. Southern blot analysis showed that two of the four responsive tumors had the EGFR gene amplification. We also examined another 73 NSCLC specimens (47 males and 26 females; 53 adenocarcinomas and 20 non-adenocarcinomas) which were not treated with gefitinib to determine whether NSCLCs with an EGFR mutation have different clinicopathological properties and/or unique genetic alterations of the other cancer-associated genes. We found that 13 (18%) of 73 tumors had a mutation of the EGFR gene, with the most being detected in female adenocarcinomas. Comparing the alterations in KRAS and P53 with the EGFR mutation, we found that 10 tumors with the KRAS mutation did not have an EGFR mutation, suggesting that each mutation occurs exclusively during the development of lung cancer. These results suggest that the mutation analysis of the EGFR gene using the specimens obtained at surgery might be useful in selecting the appropriate treatment(s) for recurrent lung cancer patients.

Kosaka, T., Y. Yatabe, et al. (2006). "Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib." Clin Cancer Res **12**(19): 5764-9.

PURPOSE: Non-small cell lung cancers carrying activating mutations in the gene for the epidermal growth factor receptor (EGFR) are highly sensitive to EGFR-specific tyrosine kinase inhibitors. However, most patients who initially respond subsequently experience disease progression while still on treatment. Part of this "acquired resistance" is attributable to a secondary mutation resulting in threonine to methionine at codon 790 (T790M) of EGFR. EXPERIMENTAL DESIGN: We sequenced exons 18 to 21 of the EGFR gene to look for secondary mutations in tumors with acquired resistance to gefitinib in 14 patients with adenocarcinomas. Subcloning or cycleave PCR was used in addition to normal sequencing to increase the sensitivity of the assay. We also looked for T790M in pretreatment samples from 52 patients who were treated with gefitinib. We also looked for secondary KRAS gene mutations because tumors with KRAS mutations are generally resistant to tyrosine kinase inhibitors. RESULTS: Seven of 14 tumors had a secondary T790M mutation. There were no other novel secondary mutations. We detected no T790M mutations in pretreatment specimens from available five tumors among these seven tumors. Patients with T790M tended to be women, never smokers, and carrying deletion mutations, but the T790M was not associated with the duration of gefitinib administration. None of the tumors had an acquired mutation in the KRAS gene. CONCLUSIONS: A secondary T790M mutation of EGFR accounted for half the tumors with acquired resistance to gefitinib in Japanese patients. Other drug-resistant secondary mutations are uncommon in the EGFR gene.

Kotsinas, A., K. Evangelou, et al. (2008). "The 3' UTR IGF2R-A2/B2 variant is associated with increased tumor growth and advanced stages in non-small cell lung cancer." Cancer Lett **259**(2): 177-85.

Normal function of insulin-like growth factor II receptor (IGF2R) gene has been associated with negative control of tumor growth in vivo and in vitro. Rare alleles at a 3' UTR short tandem repeat polymorphism of IGF2R are known to decrease transcript stability. One such allele (A2/B2) increases significantly the risk of oral squamous cell carcinoma and non-small cell lung carcinoma (NSCLC) in Caucasians. To determine potential association(s) between A2/B2 presence and development and/or progression of disease, we examined in 103 NSCLC patients, free of IGF2R allelic imbalance aberrations, the 3' UTR allelic status in relation to tumor kinetic parameters (proliferation index-PI and apoptotic index-AI) and clinicopathological data. PCR and automated sequence analyses were employed to genotype the IGF2R 3' UTR polymorphism. Given

that, oncogenic mitogens, which escape degradation by IGF2R, can also activate p53 through a DNA damage response, the patterns between p53 status and IGF2R genetic constitution were also evaluated in relation to the above parameters. The A2/B2 variant was significantly more common (p=0.005, chi2-test) in lung cancer patients (25% vs 15%). Its presence was accompanied by high cellular proliferation (p=0.028, t-test) along with increased tumor cell growth (GI=PI/AI) (p=0.022, t-test) and it was significantly found in advanced stages. Also, patients carrying the A2/B2 in their genetic constitution that exhibit aberrant p53 expression have faster growing tumors and progress more rapidly to advanced stages. In conclusion, the IGF2R-A2/B2 variant probably provides a selective advantage for NSCLC progression through increased tumor growth.

Krishnaswamy, S., R. Kanteti, et al. (2009). "Ethnic differences and functional analysis of MET mutations in lung cancer." Clin Cancer Res **15**(18): 5714-23.

PURPOSE: African Americans have higher incidence and poorer response to lung cancer treatment compared with Caucasians. However, the underlying molecular mechanisms for the significant ethnic difference are not known. The present study examines the ethnic differences in the type and frequency of MET proto-oncogene (MET) mutation in lung cancer and correlated them with other frequently mutated genes such as epidermal growth factor receptor (EGFR), KRAS2, and TP53. EXPERIMENTAL DESIGN: Using tumor tissue genomic DNA from 141 Asian, 76 Caucasian, and 66 African American lung cancer patients, exons coding for MET and EGFR were PCR amplified, and mutations were detected by sequencing. Mutation carriers were further screened for KRAS2 and TP53 mutations. Functional implications of important MET mutations were explored by molecular modeling and hepatocyte growth factor binding studies. RESULTS: Unlike the frequently encountered somatic mutations in EGFR, MET mutations in lung tumors were germline. MET-N375S, the most frequent mutation of MET, occurred in 13% of East Asians compared with none in African Americans. The frequency of MET mutations was highest among male smokers and squamous cell carcinoma. The MET-N375S mutation seems to confer resistance to MET inhibition based on hepatocyte growth factor ligand binding, molecular modeling, and apoptotic susceptibility to MET inhibitor studies. CONCLUSIONS: MET in lung cancer tissues contained nonsynonymous mutations in the semaphorin and juxtamembrane domains but not in the tyrosine kinase domain. All the MET mutations were germline. East Asians, African-Americans, and Caucasians had different MET genotypes and haplotypes. MET mutations in the semaphorin domain affected ligand binding.

Lai, J. C., Y. W. Cheng, et al. (2005). "Gender difference in estrogen receptor alpha promoter hypermethylation and its prognostic value in non-small cell lung cancer." Int J Cancer **117**(6): 974-80.

It has been documented that estrogen receptor (ER) transcription silencing due to hypermethylation is linked to the tumor progression of breast, uterine and prostate cancers. Additionally, ER hypermethylation in lung tumors has been associated with the exposure of specific carcinogens in animal study. The role of hypermethylation-induced ER transcription silencing in lung tumor progression and its prognostic value for non-small cell lung cancer (NSCLC) patients remained unclear. In our study, ER hypermethylation of 123 lung tumors and adjacent normal parts were examined by methylation-specific PCR (MSP). Estrogen receptor mRNA expression in lung tumors was determined by RT-PCR. Our data indicated that ER hypermethylation was only detected in lung tumors, but not in adjacent normal lung tissues. This suggests that ER hypermethylation may be associated with lung tumorigenesis. Among the clinical parameters studied, only gender factor was correlated with ER hypermethylation with a higher frequency of ER hypermethylation being in male patients than in female patients (58 vs. 34%, p = 0.01). After being stratified by gender and cigarette smoking status, a similarly high prevalence of ER hypermethylation was found in male smoking and nonsmoking patients (60 vs. 61%) as compared to that of female nonsmoking patients (34%). To investigate if 17-beta estradiol (E2) was responsible for such gender difference in ER hypermethylation, a lung cancer A549 cell with ER hypermethylation and without ER mRNA expression was treated with E2 of various concentrations for defined time intervals to show that an E2 treatment could restore the expression of ER mRNA and eliminate ER hypermethylation. Western blot data also showed that acetylated histone 3 and histone 4 of chromatin were increased significantly by E2 treatment. Thus, E2 can make ER mRNA re-expression by eliminating ER hypermethylation. To elucidate the prognostic value of ER hypermethylation, Kaplan-Meier analysis was carried out to show that patients with ER hypermethylation had a poorer prognosis than those without ER hypermethylation. Such prognostic prediction, however, applied only to male (p = 0.0044) patients. Cox regression analysis further showed the feasibility of ER hypermethylation as an independent prognostic factor of NSCLC (p = 0.007). It is possible that antiestrogens may have different therapeutic values for male and female lung cancer patients.

Lai, M. D., M. C. Yen, et al. (2009). "The effects of DNA formulation and administration route on cancer therapeutic efficacy with xenogenic EGFR DNA vaccine in a lung cancer animal model." Genet Vaccines Ther **7**: 2.

BACKGROUND: Tyrosine kinase inhibitor gefitinib is effective against lung cancer cells carrying mutant epidermal growth factor receptor (EGFR); however, it is not effective against lung cancer carrying normal EGFR. The breaking of immune tolerance against self epidermal growth factor receptor with active immunization may be a useful approach for the treatment of EGFR-positive lung tumors. Xenogeneic EGFR gene was demonstrated to induce antigen-specific immune response against EGFR-expressing tumor with intramuscular administration. METHODS: In order to enhance the therapeutic effect of xenogeneic EGFR DNA vaccine, the efficacy of altering routes of administration and formulation of plasmid DNA was evaluated on the mouse lung tumor (LL2) naturally overexpressing endogenous EGFR in C57B6 mice. Three different combination forms were studied, including (1) intramuscular administration of non-coating DNA vaccine, (2) gene gun administration of DNA vaccine coated on gold particles, and (3) gene gun administration of non-coating DNA vaccine. LL2-tumor bearing C57B6 mice were immunized four times at weekly intervals with EGFR DNA vaccine. RESULTS: The results indicated that gene gun administration of non-coating xenogenic EGFR DNA vaccine generated the strongest cytotoxicity T lymphocyte activity and best antitumor effects. CD8(+) T cells were essential for anti-tumor immunity as indicated by depletion of lymphocytes in vivo. CONCLUSION: Thus, our data demonstrate that administration of non-coating xenogenic EGFR DNA vaccine by gene gun may be the preferred method for treating EGFR-positive lung tumor in the future.

Lee, C. Y., H. F. Sher, et al. (2008). "Anticancer effects of tanshinone I in human non-small cell lung cancer." Mol Cancer Ther **7**(11): 3527-38.

Tanshinones are the major bioactive compounds of Salvia miltiorrhiza Bunge (Danshen) roots, which are used in many therapeutic remedies in Chinese traditional medicine. We investigated the anticancer effects of tanshinones on the highly invasive human lung adenocarcinoma cell line, CL1-5. Tanshinone I significantly inhibited migration, invasion, and gelatinase activity in macrophage-conditioned medium-stimulated CL1-5 cells in vitro and also reduced the tumorigenesis and metastasis in CL1-5-bearing severe combined immunodeficient mice. Unlike tanshinone IIA, which induces cell apoptosis, tanshinone I did not have direct cytotoxicity. Real-time quantitative PCR, luciferase reporter assay, and electrophoretic mobility shift assay revealed that tanshinone I reduces the transcriptional activity of interleukin-8, the angiogenic factor involved in cancer metastasis, by attenuating the DNA-binding activity of activator protein-1 and nuclear factor-kappaB in conditioned medium-stimulated CL1-5 cells. Microarray and pathway analysis of tumor-related genes identified the differentially expressed genes responding to tanshinone I, which may be associated with the Ras-mitogen-activated protein kinase and Rac1 signaling pathways. These results suggest that tanshinone I exhibits anticancer effects both in vitro and in vivo and that these effects are mediated at least partly through the interleukin-8, Ras-mitogen-activated protein kinase, and Rac1 signaling pathways. Although tanshinone I has a remarkable anticancer action, its potential anticoagulant effect should be noted and evaluated.

Lee, H. Y., K. H. Chun, et al. (2002). "Insulin-like growth factor binding protein-3 inhibits the growth of non-small cell lung cancer." Cancer Res **62**(12): 3530-7.

Insulin-like growth factors (IGFs) have mitogenic and antiapoptotic properties and have been implicated in the development of lung cancer. The effects of IGFs are modulated by insulin-like growth factor binding proteins (IGFBPs). This study explored the effects of IGFBP-3 on non-small cell lung cancer (NSCLC) cells after infection with an adenovirus constitutively expressing IGFBP-3 under the control of the cytomegalovirus promoter (Ad5CMV-BP3). We found that IGFs, especially IGF-I, stimulated the growth of NSCLC cells, and Ad5CMV-BP3 suppressed this IGF-I-induced NSCLC cell growth. We also found that the clonogenicity of H1299 cells in soft agar was markedly reduced by Ad5CMV-BP3. Furthermore, direct injection of Ad5CMV-BP3 into H1299 NSCLC xenografts s.c. established in athymic nude mice induced massive destruction of the tumors. Ad5CMV-BP3 did not induce detectable cytotoxicity on normal human bronchial epithelial cells, suggesting therapeutic efficacy of this virus. Ad5CMV-BP3 infection was accompanied by apoptotic cell death in vitro as detected by flow cytometry, DNA fragmentation analysis, and Western blot analysis on the expression of Bcl-2 and on the cleavage of poly(ADP-ribose) polymerase, a substrate of caspase 3. Immunofluorescence confocal microscopy was also used to show the apoptotic effect of Ad5CMV-BP3 in H1299 tumors established in nude mice. These findings indicated that IGFBP-3 was a potent inducer of apoptosis in NSCLC cells in vitro and in vivo. To

delineate the underlying mechanism, we examined the effect of IGFBP-3 on Akt/protein kinase B and glycogen synthase kinase-3beta, downstream mediators of the phosphatidylinositol 3-kinase pathway, and on mitogen-activated protein kinase (MAPK), all three of which are activated by IGF-mediated signaling pathways and have important roles in cell survival. IGFBP-3 overexpression inhibited the phosphorylation of Akt and glycogen synthase kinase-3beta and the activity of MAPK. Furthermore, IGF-I rescued the NSCLC cells from serum depletion-induced apoptosis, and this rescue was blocked in Ad5CMV-BP-3-infected H1299 NSCLC cells. Transient transfection with activated Akt or constitutively active MAPK kinase-1, an upstream activator of MAPK, partially blocked IGFBP-3-induced apoptosis of NSCLC cells. These findings suggested that the growth-regulatory effect of IGFBP-3 on NSCLC cells was attributable in part to the inhibition of the IGF-induced survival pathway. These data demonstrate the importance of IGFBP-3 in the regulation of NSCLC cell proliferation, clonogenicity, and tumor growth, suggesting that IGFBP-3 is a target for the treatment of lung cancer and that Ad5CMV-BP3 is a potential therapeutic agent.

Lee, K. H., S. W. Han, et al. (2006). "Epidermal growth factor receptor mutations and response to chemotherapy in patients with non-small-cell lung cancer." Jpn J Clin Oncol **36**(6): 344-50.

BACKGROUND: The association of epidermal growth factor receptor (EGFR) mutations with the response to conventional cytotoxic chemotherapeutic agents in non-small-cell lung cancer patients has not been investigated. We retrospectively analyzed the associations between response to chemotherapy and molecular markers associated with gefitinib responsiveness including EGFR mutations. METHODS: EGFR (exons 18, 19 and 21) and K-ras mutations (exon 2) were studied by direct sequencing and p-Erk and p-Akt expressions were studied by immunohistochemistry in archival paraffin embedded tissues. Response rate (RR) and time-to-progression (TTP) of prior chemotherapy by platinum, paclitaxel and gemcitabine were analyzed with respect to the presence of EGFR and K-ras mutations, and p-Erk and p-Akt expressions. RESULTS: Of 90 patients investigated, 75 received platinums and 45 received paclitaxel as first-line chemotherapy agents. The RRS and TTPS of platinum- and paclitaxel-containing regimens were not affected by EGFR or K-ras mutations, nor by p-Erk or p-Akt expression. Fifty-seven patients received gemcitabine as first- or second-line chemotherapy. RR was not affected by EGFR or K-ras mutations or by p-Akt expression. However, all responders to gemcitabine exhibited (+) p-Erk expression [RR 30.6% for p-Erk (+) versus 0% for p-Erk (-), P = 0.01]. TTP was not affected by EGFR or K-ras mutations or by p-Erk or p-Akt expression. CONCLUSIONS: EGFR mutations did not affect response to conventional chemotherapeutic agents, namely platinums, paclitaxel and gemcitabine. Our results also suggest that it may be undesirable to use gemcitabine in patients with tumors not expressing p-Erk.

Lee, K. H., H. S. Min, et al. (2008). "ERCC1 expression by immunohistochemistry and EGFR mutations in resected non-small cell lung cancer." Lung Cancer **60**(3): 401-7.

Expression of excision repair cross-complementation group 1 (ERCC1) is important for resistance to platinum agents. Mutations of epidermal growth factor receptor (EGFR) are related to the responsiveness to tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC). This study was performed to determine if ERCC1 expression and EGFR are related to the prognosis of resected NSCLC, and to determine if ERCC1 expression and EGFR mutations are related. We used immunohistochemistry (IHC) to evaluate ERCC1 expression in tumors from 130 patients with curatively resected NSCLC. The median H-score was used as a cut-off for ERCC1 IHC. EGFR mutations were analyzed in exons 18, 19 and 21. ERCC1 expression was detected in tumors from 80 patients (61.5%). ERCC1 was expressed more frequently in smokers and in squamous cell carcinomas. Patients with a positive ERCC1 expression survived longer than ERCC1-negative patients (median overall survival 7.6 years for ERCC1-positive vs. 4.0 years for ERCC1-negative, P=0.046). Subsequent multivariate analysis suggested that ERCC1 expression is an independent prognostic marker of longer survival (hazard ratio: 0.598, 95% confidence interval: 0.357-1.001). EGFR mutations were found in 25 patients (19.2%) but did not affect overall survival. Interestingly, EGFR mutations were more frequent in ERCC1-negative tumors (12.5% in ERCC1-positive vs. 30% in ERCC1-negative tumors, P=0.014). In conclusion, ERCC1 expression was identified as a positive prognostic marker in resected NSCLC. In addition, EGFR mutations were more frequently found in ERCC1-negative tumors.

Li, J. J., Y. Ding, et al. (2009). "The overexpression of ERCC-1 is involved in the resistance of lung cancer cells to cetuximab combined with DDP." Cancer Biol Ther **8**(20): 1914-21.

Cetuximab, an antibody against epidermal growth factor receptor, has been approved for the treatment of colorectal carcinoma and head and neck

squamous cell carcinoma. There is increasing evidence that cetuximab can reverse the resistance to irinotecan (CPT-11) and oxaliplatin. Since cisplatin (DDP) is a widely used chemotherapeutics this study examined whether cetuximab could reverse the resistance to DDP. Combined treatment with DDP and cetuximab resulted in an increase in the cytotoxicity of DDP in a DDP-sensitive lung cancer cell line (A549), but not in a DDP-resistant derivative (A549/DDP). Meantime, DDP activated the EGFR pathway in A549 cells but not in A549/DDP cells in a ligand-independent fashion. After the expression of excision repair cross-complementation group 1 (ERCC-1) protein was inhibited by small interfering RNA (siRNA), the potential of cetuximab to enhance DDP-mediated cytotoxicity was restored in A549/DDP cells. These data suggested that ERCC-1 was involved in the resistance of cetuximab combined with DDP as overexpression of ERCC-1 prohibits the activation of EGFR pathway, which would facilitate the preselection of lung cancer patients for the treatment of cetuximab combined with DDP.

Li, T., Y. H. Ling, et al. (2008). "Tumor dependence on the EGFR signaling pathway expressed by the p-EGFR:p-AKT ratio predicts erlotinib sensitivity in human non-small cell lung cancer (NSCLC) cells expressing wild-type EGFR gene." J Thorac Oncol **3**(6): 643-7.

INTRODUCTION: This study was undertaken to identify molecular determinants of tumor dependency on the epidermal growth factor receptor (EGFR) signaling pathway for predicting clinical benefit from erlotinib monotherapy in non-small cell lung cancer (NSCLC) patients with tumors expressing wild-type EGFR gene. METHODS: The effect of erlotinib on the total and phosphorylated protein expression of EGFR and key downstream signaling molecules was determined by immunoblots in a panel of NSCLC cells expressing wild-type EGFR gene. The parameters that correlate with cell sensitivity and resistance to erlotinib was analyzed. RESULTS: Individual assessment of total or phosphorylated protein expression of EGFR or a downstream signaling molecule does not correlate with sensitivity to erlotinib in these NSCLC tumors. Resistance of NSCLC cells to erlotinib is associated with failed inhibition of at least one phosphorylated downstream signaling molecule. The dependency of NSCLC cells on the activated EGFR axis was measured by the ratio of p-EGFR to a phosphorylated downstream protein. A high ratio should indicate that activation of a downstream signaling molecule primarily results from the activation of upstream EGFR; and a low ratio should indicate that activation of a downstream signaling molecule primarily results from the activation of a upstream receptors other than EGFR. The p-EGFR:p-AKT ratio was 10-fold higher in erlotinib-sensitive cells than erlotinib-resistant cells ($p = 0.03$). It was the best predictor of erlotinib sensitivity among all parameters analyzed in this panel of NSCLC cell lines. CONCLUSIONS: The p-EGFR:p-AKT ratio deserves further investigation as a predictive parameter for clinical response to erlotinib in NSCLC tumors expressing wild-type EGFR gene.

Liang, C. H., L. F. Liu, et al. (2004). "Action of solamargine on TNFs and cisplatin-resistant human lung cancer cells." Biochem Biophys Res Commun **322**(3): 751-8.

A loss of TNF receptors expression has been found in advanced lung cancers, and human A549 lung adenocarcinoma cells are resistant to the cytotoxic effects of TNF-alpha and cisplatin. Here, the mechanisms of the drug resistance of A549 were extensively studied by gene modulation of the cells by solamargine (SM) which was isolated from Solanum incanum herb. SM induced morphological changes of chromatin condensation, DNA fragmentation, and sub-G(1) peak in a DNA histogram of A549 cells, indicating cell death by apoptosis. SM elevated the expressions of TNF-R1 and -R2 and overcame the resistance of A549 cells to TNF-alpha and -beta. The recruitment of TRADD, FADD, and activation of caspase-8 and -3 in SM-treated A549 cells evidenced the activation of TNFRs signal transduction. In addition, release of cytochrome c from mitochondria, down-expression of Bcl-2 and Bcl-x(L), up-regulation of Bax, and caspase-9 activities were observed in SM-treated A549 cells. Combinational treatment of SM and cisplatin synergistically enhanced caspase-8, -9, and -3 activities in A549 cells. Thus, SM sensitizes A549 cells through TNFRs and mitochondria-mediated pathways and may have anticancer potential against TNFs- and cisplatin-resistance lung cancer cells.

Liang, C. H., L. Y. Shiu, et al. (2008). "Solamargine enhances HER2 expression and increases the susceptibility of human lung cancer H661 and H69 cells to trastuzumab and epirubicin." Chem Res Toxicol **21**(2): 393-9.

We have previously demonstrated that solamargine (SM), the major steroidal glycoalkaloid extracted from Chinese herb Solanum plants, reveals down-regulation of HER2 and up-regulation of Fas and tumor necrosis factor receptor (TNFR) expressions, triggers the mitochondria-mediated cell apoptosis pathway, and sensitizes human nonsmall cell lung cancer (NSCLC) H441 and A549 adenocarcinoma cells to chemotherapy. The present study shows that SM enhances HER2 expression in

NSCLC large cell carcinoma H661 and small cell lung cancer (SCLC) H69 cells and may increase the susceptibility of the cells to trastuzumab, the humanized anti-HER2 antibody. The combinational treatment of SM and trastuzumab synergistically augments and inhibits H661 and H69 cell proliferation. After treatment with SM, coexpression of HER2 and topoisomerase IIalpha (TOP2A) H661 and H69 cells is more sensitive to the TOP2 inhibitor, epirubicin. The combinatory use of low concentrations of SM with the low-toxic epirubicin accelerated greater apoptotic cell death than each drug did alone in H661 and H69 cells. Relevant studies have shown that HER2 overexpressing cancer cells are more resistant than HER2 low-expressing cells to the chemotherapeutic agent and tumor necrosis factor-induced apoptosis. These investigations have indicated that HER2 overexpression does not suffice to induce intrinsic and pleomorphic drug resistance. The data presented herein suggest that the expression of HER2 did not influence the SM-induced apoptosis of different types of lung cancer cells and that the SM up-regulation of HER2 and TOP2A expressions simultaneously augmented trastuzumab and epirubicin-induced deaths of lung cancer H661 and H69 cells.

Lim, E. H., S. L. Zhang, et al. (2009). "Using whole genome amplification (WGA) of low-volume biopsies to assess the prognostic role of EGFR, KRAS, p53, and CMET mutations in advanced-stage non-small cell lung cancer (NSCLC)." J Thorac Oncol **4**(1): 12-21.

BACKGROUND: Progression of non-small cell lung cancer (NSCLC) from early- to late-stage may signify the accumulation of gene mutations. An advanced-stage tumor's mutation profile may also have prognostic value, guiding treatment decisions. Mutation detection of multiple genes is limited by the low amount of deoxyribonucleic acid extracted from low-volume diagnostic lung biopsies. We explored whole genome amplification (WGA) to enable multiple molecular analyses. METHODS: Eighty-eight advanced-stage NSCLC patients were enrolled. Their low-volume lung biopsies underwent WGA before direct sequencing for epidermal growth factor receptor (EGFR), KRAS (rat sarcoma virus), p53, and CMET (mesenchymal-epithelial transition factor) mutations. Overall survival impact was examined. Surgically-resected tumors from 133 early-stage NSCLC patients were sequenced for EGFR, KRAS and p53 mutations. We compared the mutation frequencies of both groups. RESULTS: It is feasible for low-volume lung biopsies to undergo WGA for mutational analysis. KRAS and CMET mutations have a deleterious effect on overall survival, hazard ratios 5.05 (p = 0.009) and 23.65 (p = 0.005), respectively. EGFR and p53 mutations, however, do not have a survival impact. There also does not seem to be significant differences in the frequency of mutations in EGFR, KRAS, and p53 between early- and advanced-stage disease: 20% versus 24% (p = 0.48), 29% versus 27% (p = 0.75), 10% versus 6% (p = 0.27), respectively. CONCLUSIONS: In advanced-stage NSCLC, KRAS, and CMET mutations suggest poor prognosis, whereas EGFR and p53 mutations do not seem to have survival impact. Mutations in EGFR, KRAS and p53 are unlikely to be responsible for the progression of NSCLC from early- to late-stage disease. WGA may be used to expand starting deoxyribonucleic acid from low-volume lung biopsies for further analysis of advanced-stage NSCLC.

Liu, L. F., C. H. Liang, et al. (2004). "Action of solamargine on human lung cancer cells--enhancement of the susceptibility of cancer cells to TNFs." FEBS Lett **577**(1-2): 67-74.

Solamargine (SM), isolated from Solanum incanum herb, displayed a superior cytotoxicity in four human lung cancer cell lines. The half-inhibitory concentrations (IC50), of the cell viability assay for H441, H520, H661 and H69 cells were 3, 6.7, 7.2 and 5.8 microM, respectively. SM-induced apoptosis of these cells by PS externalization in a dose-dependent manner and increased sub-G1 fraction were observed. Quenching of the expression of tumor necrosis factor receptors (TNFRs) during the progress of human lung carcinogenesis has been previously reported. SM may induce cell apoptosis via modulating the expression of TNFRs and their subsequent TRADD/FADD signal cascades. Subsequently, SM treatment increased the binding activities of TNF-alpha and TNF-beta to the lung cancers, and the intrinsic TNFs-resistant cancer cells became susceptible to TNF-alpha and -beta. In addition, SM caused release of cytochrome c, downregulation of anti-apoptotic Bcl-2 and Bcl-xL, increase of caspase-3 activity, and DNA fragmentation. Thus, SM could modulate the expressions of TNFRs and Bcl-2, and might be a potential anticancer agent for TNFs and Bcl-2 related resistance of human lung cancer cells.

Liu, L. Z., J. Fang, et al. (2005). "Apigenin inhibits expression of vascular endothelial growth factor and angiogenesis in human lung cancer cells: implication of chemoprevention of lung cancer." Mol Pharmacol **68**(3): 635-43.

Apigenin is a natural dietary flavonoid. It has recently been shown to have anticancer effects on prostate and ovarian cancer cells. However, the molecular basis of the effect of apigenin on cancer cells remains to be elucidated. In this study, we found

that apigenin inhibited A549 lung cancer cell proliferation and vascular endothelial growth factor (VEGF) transcriptional activation in a dose-dependent manner. In an attempt to understand the mechanism of apigenin-inhibited VEGF expression, we found that apigenin inhibited VEGF transcriptional activation through the hypoxia-inducible factor 1 (HIF-1) binding site and specifically decreased HIF-1alpha but not HIF-1beta subunit expression in the cells. In our efforts to understand the signaling pathway that mediates VEGF transcriptional activation, we found that apigenin inhibited AKT and p70S6K1 activation. When testing the effect of apigenin in vivo, we found that apigenin significantly inhibited tumor growth in nude mice. Apigenin inhibited HIF-1alpha and VEGF expression in the tumor tissues, suggesting an inhibitory effect of apigenin on angiogenesis. To confirm this, we showed that apigenin inhibited angiogenesis in nude mice using the Matrigel assay. HIF-1alpha and VEGF are well known inducers of angiogenesis. Our data suggested that apigenin may inhibit human lung cancer angiogenesis by inhibiting HIF-1alpha and VEGF expression, thus providing a novel explanation for the anticancer action of apigenin.

Liu, X., P. Yue, et al. (2004). "Death receptor regulation and celecoxib-induced apoptosis in human lung cancer cells." J Natl Cancer Inst **96**(23): 1769-80.

BACKGROUND: Celecoxib, a cyclooxygenase 2 inhibitor, has chemopreventive and therapeutic activities toward lung cancer and other epithelial malignancies. Celecoxib can induce apoptosis in various cancer cell lines through a mechanism that is independent of its cyclooxygenase 2 inhibitory activity but is otherwise largely uncharacterized. We investigated the mechanism of celecoxib-induced apoptosis further. METHODS: All experiments were conducted in human non-small-cell lung carcinoma (NSCLC) cell lines; results in celecoxib-treated and untreated cells were compared. Cell survival was assessed with a sulforhodamine B assay. Apoptosis was assessed by DNA fragmentation with an enzyme-linked immunosorbent assay, by terminal deoxynucleotidyltransferase-mediated dUTP nick-end-labeling (TUNEL) assay, and by western blot analysis of caspase activation. Death receptor gene and protein expression was detected by northern and western blot analysis, respectively. Gene silencing was achieved with small interfering RNA (siRNA) technology. RESULTS: Celecoxib treatment decreased cell survival, activated caspase cascades, and increased DNA fragmentation, all of which were abrogated when caspase 8 expression was silenced with caspase 8 siRNA. Celecoxib treatment induced the expression of death receptors, particularly that of DR5. Overexpression of a dominant negative Fas-associated death domain mutant, but not of BCL2, reduced the level of celecoxib-induced apoptosis, and silencing of DR5 expression by DR5 siRNA suppressed celecoxib-induced caspase 8 activation and apoptosis. Combination treatment with celecoxib and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induced additional apoptosis. For example, survival of A549 cells was decreased with 50 muM celecoxib alone by 38.7% (95% confidence interval [CI] = 35.2% to 42.2%), with TRAIL alone by 29.3% (95% CI = 25.1% to 33.6%), but with their combination by 77.5% (95% CI = 74.5% to 79.5%), a greater than additive effect. CONCLUSION: Celecoxib appears to induce apoptosis in human NSCLC through the extrinsic death receptor pathway.

Lonardo, F., K. H. Dragnev, et al. (2002). "Evidence for the epidermal growth factor receptor as a target for lung cancer prevention." Clin Cancer Res **8**(1): 54-60.

PURPOSE: There is a need to identify lung cancer prevention mechanisms. All-trans-retinoic acid (RA) was reported previously to inhibit N-nitrosamine-4-(methylnitrosamino)-1-(3 pyridyl)-1-butanone (NNK) carcinogenic transformation of BEAS-2B human bronchial epithelial cells (J. Langenfeld et al., Oncogene, 13: 1983-1990, 1996). This study was undertaken to identify pathways targeted during this chemoprevention. Experimental Design: Because epidermal growth factor receptor (EGFR) overexpression is frequent in non-small cell lung cancers (NSCLC) and bronchial preneoplasia, BEAS-2B cells, carcinogen-transformed BEAS-2B(NNK) cells, and retinoid chemoprevented BEAS-2B(NNK RA) cells were each examined for EGFR expression. Whether RA treatment regulated directly EGFR expression or reporter plasmid activity was studied. RA effects on epidermal growth factor (EGF) induction of EGFR-phosphotyrosine levels, cyclin D1 expression and mitogenesis were examined in BEAS-2B cells. RESULTS: Findings reveal that NNK-mediated transformation of BEAS-2B cells increased EGFR expression. RA treatment repressed EGFR expression and reporter plasmid activity in these cells. This treatment reduced EGF-dependent mitogenesis as well as EGFR-associated phosphotyrosine levels and cyclin D1 expression. These findings extend prior work by highlighting EGFR as a chemoprevention target in the lung. Notably, RA treatment prevented transformation as well as outgrowth of EGFR overexpressing bronchial epithelial cells, despite NNK exposure. After acute NNK exposure, p53-induced species that appear after DNA damage or oxidative stress were evident before an observed increase in EGFR expression. CONCLUSIONS: These findings indicate how effective chemoprevention prevents

carcinogenic transformation of bronchial epithelial cells when repair of genomic damage does not select against EGFR overexpressing cells. This implicates EGFR as a chemoprevention target in the carcinogen-exposed bronchial epithelium.

Lord, R. V., J. Brabender, et al. (2002). "Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer." Clin Cancer Res **8**(7): 2286-91.

PURPOSE: Overexpression of the excision repair cross-complementing 1 (ERCC1) gene, which is crucial in the repair of cisplatin (CDDP)-DNA adducts, is reported to negatively influence the effectiveness of CDDP-based therapy for gastric and ovarian cancers. Recent evidence indicates that Gemcitabine (Gem) may modulate ERCC1 nucleotide excision repair activity, and down-regulation of DNA repair activity by ERCC1 antisense RNA reportedly inhibits synergism of CDDP/Gem. We investigated whether ERCC1 mRNA expression levels were associated with clinical outcomes after treatment with a combination Gem/CDDP regimen for patients with advanced stage non-small cell lung cancer (NSCLC). EXPERIMENTAL DESIGN: Response and survival were correlated with the level of ERCC1 expression in 56 patients with advanced (stage IIIb or IV) NSCLC treated as part of a multicenter randomized trial with Gem 1250 mg/m(2) days 1 and 8 plus CDDP 100 mg/m(2) on day 1 every 3 weeks. mRNA was isolated from paraffin-embedded pretreatment primary tumor specimens, and relative expression levels of ERCC1/beta-actin were measured using a quantitative reverse transcription-PCR (Taqman) system. RESULTS: ERCC1 expression was detectable in all tumors. There were no significant differences in ERCC1 levels by gender, age, performance status, weight loss, or tumor stage. The overall response rate was 44.7%. There were no significant associations between ERCC1 expression and response. Median overall survival was significantly longer in patients with low ERCC1 expression tumors (61.6 weeks; 95% confidence interval, 42.4-80.7 weeks) compared to patients with high expression tumors (20.4 weeks, 95% confidence interval, 6.9-33.9 weeks). ERCC1 expression, Eastern Cooperative Oncology Group performance status, and presence of weight loss were significant prognostic factors for survival in a Cox proportional hazards multivariable analysis. CONCLUSIONS: These data suggest that ERCC1 expression is a predictive factor for survival after CDDP/Gem therapy in advanced NSCLC. Although there was a trend toward decreased response with high ERCC1 mRNA levels, this difference failed to reach statistical significance. This result may reflect the impact of Gem and the requirement for ERCC1 expression for CDDP/Gem synergism or may be attributable to the relatively small patient sample size in this study. Prospective studies of ERCC1 as a predictive marker for activity of CDDP-based regimens in NSCLC are warranted.

Lynch, T. J., D. W. Bell, et al. (2004). "Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib." N Engl J Med **350**(21): 2129-39.

BACKGROUND: Most patients with non-small-cell lung cancer have no response to the tyrosine kinase inhibitor gefitinib, which targets the epidermal growth factor receptor (EGFR). However, about 10 percent of patients have a rapid and often dramatic clinical response. The molecular mechanisms underlying sensitivity to gefitinib are unknown. METHODS: We searched for mutations in the EGFR gene in primary tumors from patients with non-small-cell lung cancer who had a response to gefitinib, those who did not have a response, and those who had not been exposed to gefitinib. The functional consequences of identified mutations were evaluated after the mutant proteins were expressed in cultured cells. RESULTS: Somatic mutations were identified in the tyrosine kinase domain of the EGFR gene in eight of nine patients with gefitinib-responsive lung cancer, as compared with none of the seven patients with no response (P<0.001). Mutations were either small, in-frame deletions or amino acid substitutions clustered around the ATP-binding pocket of the tyrosine kinase domain. Similar mutations were detected in tumors from 2 of 25 patients with primary non-small-cell lung cancer who had not been exposed to gefitinib (8 percent). All mutations were heterozygous, and identical mutations were observed in multiple patients, suggesting an additive specific gain of function. In vitro, EGFR mutants demonstrated enhanced tyrosine kinase activity in response to epidermal growth factor and increased sensitivity to inhibition by gefitinib. CONCLUSIONS: A subgroup of patients with non-small-cell lung cancer have specific mutations in the EGFR gene, which correlate with clinical responsiveness to the tyrosine kinase inhibitor gefitinib. These mutations lead to increased growth factor signaling and confer susceptibility to the inhibitor. Screening for such mutations in lung cancers may identify patients who will have a response to gefitinib.

Macedo, J. E., A. M. Costa, et al. (2007). "Genetic alterations in lung cancer: assessing limitations in routine clinical use." Rev Port Pneumol **13**(1): 9-34.

Lung cancer is the most frequent cause of cancer mortality worldwide, responsible for approximately 1.1 million deaths per year. Median

survival is short, both as most tumours are diagnosed at an advanced stage and because of the limited efficacy of available treatments. The development of tumour molecular gene- tics carries the promise of altering this state of affairs, as it should lead to a more precise classification of tumours, identify specific molecular targets for therapy and, above all, allow the development of new methods for early diagnosis. Despite numerous studies demonstrating the usefulness of molecular genetic techniques in the study of lung cancer, its routine clinical use in Portugal has, however, been limited. In this study, we used a p53 mutation screen in multi- ple clinical samples from a series of lung cancer patients to attempt to identify the main practical limitations to the integration of molecular genetics in routine clinical practice. Our results suggest that the main limiting factor is the availability of samples with good quality DNA; a problem that could be overcome by alterations in common sample collection and storage procedures.

Mahaffey, C. M., A. M. Davies, et al. (2007). "Schedule-dependent apoptosis in K-ras mutant non-small-cell lung cancer cell lines treated with docetaxel and erlotinib: rationale for pharmacodynamic separation." Clin Lung Cancer **8**(9): 548-53.

BACKGROUND: Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) given concurrently with chemotherapy in 4 large randomized clinical trials did not improve patient outcomes compared with chemotherapy alone in advanced non-small-cell lung cancer (NSCLC). We hypothesized that the lack of benefit resulted from a negative interaction between chemotherapy and EGFR TKIs. MATERIALS AND METHODS: Herein, we report the cell cycle and apoptotic effects of treatment with erlotinib and docetaxel in the NSCLC cell lines A549 and Calu-1, both of which are mutant for K-ras and wild-type for EGFR. RESULTS: Treatment with erlotinib resulted in accumulation of cells in G(1) phase in A549 cells, with no evidence of apoptosis. Docetaxel treatment led to apoptosis as assessed by increased sub-G1 DNA content and cleavage of caspase 3 and poly (ADP-ribose) polymerase. The sequence of docetaxel followed by erlotinib resulted in significantly enhanced apoptosis compared with single-agent docetaxel in both cell lines. However, in the reverse sequence of erlotinib followed by docetaxel, a reduction of apoptosis was observed. We hypothesize that cell cycle arrest induced by erlotinib accounts for these findings in the presence of wild-type EGFR and that pharmacodynamic separation of the 2 drug classes will ameliorate these effects. CONCLUSION: These studies provide a rationale for intermittent dosing of EGFR TKIs with chemotherapy in order to enhance cytotoxicity.

Maheswaran, S., L. V. Sequist, et al. (2008). "Detection of mutations in EGFR in circulating lung-cancer cells." N Engl J Med **359**(4): 366-77.

BACKGROUND: The use of tyrosine kinase inhibitors to target the epidermal growth factor receptor gene (EGFR) in patients with non-small-cell lung cancer is effective but limited by the emergence of drug-resistance mutations. Molecular characterization of circulating tumor cells may provide a strategy for noninvasive serial monitoring of tumor genotypes during treatment. METHODS: We captured highly purified circulating tumor cells from the blood of patients with non-small-cell lung cancer using a microfluidic device containing microposts coated with antibodies against epithelial cells. We performed EGFR mutational analysis on DNA recovered from circulating tumor cells using allele-specific polymerase-chain-reaction amplification and compared the results with those from concurrently isolated free plasma DNA and from the original tumor-biopsy specimens. RESULTS: We isolated circulating tumor cells from 27 patients with metastatic non-small-cell lung cancer (median number, 74 cells per milliliter). We identified the expected EGFR activating mutation in circulating tumor cells from 11 of 12 patients (92%) and in matched free plasma DNA from 4 of 12 patients (33%) (P=0.009). We detected the T790M mutation, which confers drug resistance, in circulating tumor cells collected from patients with EGFR mutations who had received tyrosine kinase inhibitors. When T790M was detectable in pretreatment tumor-biopsy specimens, the presence of the mutation correlated with reduced progression-free survival (7.7 months vs. 16.5 months, P<0.001). Serial analysis of circulating tumor cells showed that a reduction in the number of captured cells was associated with a radiographic tumor response; an increase in the number of cells was associated with tumor progression, with the emergence of additional EGFR mutations in some cases. CONCLUSIONS: Molecular analysis of circulating tumor cells from the blood of patients with lung cancer offers the possibility of monitoring changes in epithelial tumor genotypes during the course of treatment.

Mano, Y., K. Takahashi, et al. (2007). "Fibroblast growth factor receptor 1 oncogene partner as a novel prognostic biomarker and therapeutic target for lung cancer." Cancer Sci **98**(12): 1902-13.

To screen candidate molecules that might be useful as diagnostic biomarkers or for development of novel molecular-targeting therapies, we previously

carried out gene-expression profile analysis of 101 lung carcinomas and detected an elevated expression of FGFR1OP (fibroblast growth factor receptor 1 oncogene partner) in the majority of lung cancers. Immunohistochemical staining using tumor tissue microarrays consisting of 372 archived non-small cell lung cancer (NSCLC) specimens revealed positive staining of FGFR1OP in 334 (89.8%) of 372 NSCLCs. We also found that the high level of FGFR1OP expression was significantly associated with shorter tumor-specific survival times ($P < 0.0001$ by log-rank test). Moreover, multivariate analysis determined that FGFR1OP was an independent prognostic factor for surgically treated NSCLC patients ($P < 0.0001$). Treatment of lung cancer cells, in which endogenous FGFR1OP was overexpressed, using FGFR1OP siRNA, suppressed its expression and resulted in inhibition of the cell growth. Furthermore, induction of FGFR1OP increased the cellular motility and growth-promoting activity of mammalian cells. To investigate its function, we searched for FGFR1OP-interacting proteins in lung cancer cells and identified ABL1 (Abelson murine leukemia viral oncogene homolog 1) and WRNIP1 (Werner helicase interacting protein 1), which was known to be involved in cell cycle progression. FGFR1OP significantly reduced ABL1-dependent phosphorylation of WRNIP1 and resulted in the promotion of cell cycle progression. Because our data imply that FGFR1OP is likely to play a significant role in lung cancer growth and progression, FGFR1OP should be useful as a prognostic biomarker and probably as a therapeutic target for lung cancer.

Marchetti, A., M. Milella, et al. (2009). "Clinical implications of KRAS mutations in lung cancer patients treated with tyrosine kinase inhibitors: an important role for mutations in minor clones." Neoplasia **11**(10): 1084-92.

Mutations inducing resistance to anti-epidermal growth factor receptor (EGFR) therapy may have a clinical impact even if present in minor cell clones which could expand during treatment. We tested this hypothesis in lung cancer patients treated with tyrosine kinase inhibitors (TKIs). Eighty-three patients with lung adenocarcinoma treated with erlotinib or gefitinib were included in this study. The mutational status of KRAS and EGFR was investigated by direct sequencing (DS). KRAS mutations were also assessed by mutant-enriched sequencing (ME-sequencing). DS detected KRAS mutations in 16 (19%) of 83 tumors; ME-sequencing identified all the mutations detected by DS but also mutations in minor clones of 14 additional tumors, for a total of 30 (36%) of 83. KRAS mutations assessed by DS and ME-sequencing significantly correlated with resistance to TKIs ($P = .04$ and $P = .004$, respectively) and significantly affected progression-free survival (PFS) and overall survival (OS). However, the predictive power of mutations assessed by ME-sequencing was higher than that obtained by DS (hazard ratio [HR] = 2.82, $P = .0001$ vs HR = 1.98, $P = .04$, respectively, for OS; HR = 2.52, $P = .0005$ vs HR = 2.21, $P = .007$, respectively, for PFS). Survival outcome of patients harboring KRAS mutations in minor clones, detected only by ME-sequencing, did not differ from that of patients with KRAS mutations detected by DS. Only KRAS mutations assessed by ME-sequencing remained an independent predictive factor at multivariate analysis. KRAS mutations in minor clones have an important impact on response and survival of patients with lung adenocarcinoma treated with EGFR-TKI. The use of sensitive detection methods could allow to more effectively identify treatment-resistant patients.

Marsit, C. J., D. H. Kim, et al. (2005). "Hypermethylation of RASSF1A and BLU tumor suppressor genes in non-small cell lung cancer: implications for tobacco smoking during adolescence." Int J Cancer **114**(2): 219-23.

The putative tumor suppressors RASSF1A and BLU are mapped adjacent to one another on chromosome 3p21.3, a region frequently deleted in lung cancer. These genes are often inactivated by promoter hypermethylation, but the association of this inactivation with clinical features of the disease or with carcinogen exposure has been poorly studied. Early age starting smoking has been hypothesized as an independent risk factor for lung cancer, and mechanistically, adolescence may constitute a critical period for tobacco carcinogen exposure. To study the relationship of tobacco smoke exposure with hypermethylation of RASSF1A and BLU, methylation-specific PCR was performed on a case series study of incident, surgically resected non-small cell lung cancer (NSCLC), and the prevalence of this alteration was examined in relation to clinical and exposure information collected on the patients. Hypermethylation of the RASSF1A promoter occurred in 47% (83/178) and of the BLU promoter in 43% (68/160) of NSCLC tumors examined. There was no significant association between methylation of these 2 genes, but methylation of either of these genes tended to occur more often in the adenocarcinoma (AC) histology compared to squamous cell carcinoma (SCC). Controlling for pack-years smoked, age, gender and histology, starting smoking under age 18 was significantly related to RASSF1A methylation [prevalence ratio (PR) = 1.6, 95% confidence interval [CI] = 1.1-2.3]. These results indicate that starting smoking under age 18 is an independent risk for

RASSF1A hypermethylation, thus identifying a molecular alteration related to the epidemiologic effect of teenage smoking as a lung cancer risk.

Masago, K., S. Fujita, et al. (2008). "Accuracy of epidermal growth factor receptor gene mutation analysis by direct sequencing method based on small biopsy specimens from patients with non-small cell lung cancer: analysis of results in 19 patients." Int J Clin Oncol **13**(5): 442-6.

BACKGROUND: The importance of an epidermal growth factor receptor (EGFR) gene mutation has been recognized in patients with non-small cell lung cancer (NSCLC), and many reports have indicated that the presence of somatic mutations in the EGFR gene is a strong predictor of both clinical and in vitro sensitivity to EGFR tyrosine kinase inhibitors; thus necessitating the standardization of a mutation screening system based on the sources of tissue samples. METHODS: In this study, we compared the results of EGFR mutation analyses in 19 small biopsy specimens with results obtained in surgical materials from the same patients with NSCLC. We used a laser microdissection method and a direct sequencing method, and we confirmed the accuracy of EGFR mutation analysis with small biopsy specimens. RESULTS: The results obtained from the biopsy specimens were identical to those obtained from the surgical materials in 18 of the 19 patients analyzed. For the 1 patient in whom the results obtained from the two sets of materials were not identical, the number of cancer cells in one bronchoscopic specimen was insufficient to perform analyses of all three exons of interest (i.e., exons 18, 19, and 21), and so only exon 19 was sequenced, and no mutation was demonstrated. CONCLUSION: We conclude that satisfactory accuracy can be achieved by the genomic analysis of a small biopsy specimen from a patient with NSCLC and we note that it is possible to conduct prospective clinical trials that include patient assignment for treatment based on the results obtained.

Mazieres, J., B. He, et al. (2004). "Wnt inhibitory factor-1 is silenced by promoter hypermethylation in human lung cancer." Cancer Res **64**(14): 4717-20.

Aberrant activation of the Wingless-type (Wnt) signaling pathway is associated with a variety of human cancers, and we recently reported the importance of aberrant Wnt signaling in lung cancer. On the other hand, inhibition of Wnt signaling suppresses growth in numerous cell types. Wnt inhibitory factor-1 (WIF-1) is a secreted antagonist that can bind Wnt in the extracellular space and inhibit Wnt signaling. Recently, down-regulation of WIF-1 has been reported in several human cancers. To discover the mechanism of WIF-1 silencing in lung cancer, we first identified the human WIF-1 promoter and subsequently examined the methylation status in the CpG islands. By using methylation-specific PCR and sequence analysis after bisulfite treatment, we demonstrate here frequent CpG island hypermethylation in the functional WIF-1 promoter region. This hypermethylation correlates with its transcriptional silencing in human lung cancer cell lines. Moreover, treatment with 5-aza-2'-deoxycytidine restores WIF-1 expression. We then studied WIF-1 expression in 18 freshly resected lung cancers, and we show a down-regulation in 15 of them (83%). This silencing also correlates with WIF-1 promoter methylation. Our results suggest that methylation silencing of WIF-1 is a common and likely important mechanism of aberrant activation of the Wnt signaling pathway in lung cancer pathogenesis, raising its therapeutic interest.

Mehrotra, J., M. Vali, et al. (2004). "Very high frequency of hypermethylated genes in breast cancer metastasis to the bone, brain, and lung." Clin Cancer Res **10**(9): 3104-9.

PURPOSE: Most often it is not the primary tumor, but metastasis to distant organs that results in the death of breast cancer patients. To characterize molecular alterations in breast cancer metastasis, we investigated the frequency of hypermethylation of five genes (Cyclin D2, RAR-beta, Twist, RASSF1A, and HIN-1) in metastasis to four common sites: lymph node, bone, brain, and lung. EXPERIMENTAL DESIGN: Methylation-specific PCR for the five genes was performed on DNA extracted from archival paraffin-embedded specimens of paired primary breast cancer and its lymph nodes (LN) metastasis (n = 25 each); in independent samples of metastasis to the bone (n = 12), brain (n = 8), and lung (n = 10); and in normal bone, brain, and lung (n = 22). RESULTS: No hypermethylation was detected in the five genes in the normal host tissues. In paired samples, LN metastasis had a trend of higher prevalence of methylation compared with the primary breast carcinoma for all five genes with significance for HIN-1 ($P = 0.04$). Compared with the primary breast carcinomas, all five genes had higher methylation frequencies in the bone, brain, and lung metastasis, with HIN-1 and RAR-beta methylation being significantly higher ($P < 0.01$) in each group. Loss of expression of all five genes correlated, with a few exceptions, to hypermethylation of their promoter sequences in metastatic carcinoma cells microdissected from LNs. CONCLUSION: The frequent presence of hypermethylated genes in locoregional and distant metastasis could render them particularly susceptible to therapy targeted toward gene reactivation combining demethylating agents,

histone deacetylase inhibitors, and/or differentiating agents.

Mi, J., X. Zhang, et al. (2008). "RNA aptamer-targeted inhibition of NF-kappa B suppresses non-small cell lung cancer resistance to doxorubicin." Mol Ther **16**(1): 66-73.

Due to the prevalence of tumor chemoresistance, the clinical response of advanced non-small cell lung cancer (NSCLC) to chemotherapy is poor. We suppressed tumor resistance to doxorubicin (Dox) in A549 cells, a human NSCLC cell line, both in vitro and in vivo in a lung tumor xenograft model, using a novel adenoviral expression system to deliver an RNA aptamer (A-p50) that specifically inhibits nuclear factor-kappaB (NF-kappaB) activation. By achieving selective, targeted, and early inhibition of NF-kappaB activity, we demonstrate that NF-kappaB plays a critical role in Dox-induced chemoresistance by regulating genes involved in proliferation (Ki-67), response to DNA damage (GADD153), antiapoptosis (Bcl-XL), and pH regulation (CA9). This Dox-induced NF-kappaB activation and subsequent chemoresistance is dependent on expression of p53. We also demonstrate that NF-kappaB promotes angiogenesis in the presence of Dox via the hypoxia-inducible factor-1alpha/vascular endothelial growth factor (HIF-1alpha/VEGF) pathway, revealing a previously unknown mechanism of NSCLC resistance to Dox. These studies provide important insights into the mechanisms of Dox-induced chemoresistance, and they demonstrate a novel, effective, and clinically practical strategy for interfering with these processes.

Mitsudomi, T., T. Kosaka, et al. (2005). "Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence." J Clin Oncol **23**(11): 2513-20.

PURPOSE: To evaluate the relationship between mutations of the epidermal growth factor receptor (EGFR) gene and the effectiveness of gefitinib treatment in patients with recurrent lung cancer after pulmonary resection. PATIENTS AND METHODS: We sequenced exons 18-21 of the EGFR gene using total RNA extracted from 59 patients with lung cancer who were treated with gefitinib for recurrent lung cancer. Gefitinib effectiveness was evaluated by both imaging studies and change in serum carcinoembryonic antigen (CEA) levels. RESULTS: EGFR mutations were found in 33 patients (56%). Of these mutations, 17 were deletions around codons 746-750 and 15 were point mutations (12 at codon 858, three at other codons), and one was an insertion. EGFR mutations were significantly more prevalent in females, adenocarcinoma, and never-smokers. Gefitinib treatment resulted in tumor shrinkage and/or CEA decrease to less than half of the baseline level in 26 patients, tumor growth and/or CEA elevation in 24 patients, and gefitinib effect was not assessable in nine patients. Female, never-smoking patients with adenocarcinoma tended to respond better to gefitinib treatment. Gefitinib was effective in 24 of 29 patients with EGFR mutations, compared with two of 21 patients without mutations ($P < .0001$). Of note, del746-750 might be superior to L858R mutations for prediction of gefitinib response. Patients with EGFR mutations survived for a longer period than those without the mutations after initiation of gefitinib treatment ($P = .0053$). CONCLUSION: EGFR mutations were a good predictor of clinical benefit of gefitinib in this setting.

Miyanaga, A., A. Gemma, et al. (2008). "E-cadherin expression and epidermal growth factor receptor mutation status predict outcome in non-small cell lung cancer patients treated with gefitinib." Oncol Rep **19**(2): 377-83.

It is known that an epidermal growth factor receptor (EGFR) gene mutation(s) is present in a percentage of non-small cell lung cancers (NSCLCs). Gefitinib, an inhibitor of the tyrosine kinase activity of EGFR, is effective on most of them. The EGFR mutation status alone cannot fully predict the response to gefitinib and the prognosis for the patients. We hypothesized that information on the expression levels of phosphorylated-EGFR and -Akt, and E-cadherin, alone or in combination with information on the EGFR mutation, may refine our ability of prediction. We investigated 24 NSCLCs that had recurred after surgery and were treated with gefitinib. Specimens resected by surgery were subjected to the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp reaction to determine the EGFR mutation status, and to immunohistochemical staining of phosphorylated-EGFR and -Akt, and E-cadherin to determine their expression levels. The EGFR mutation status was predictive of responsive disease (complete response: CR + partial response: PR) and controlled disease (CR + PR + stable disease: SD). Positive E-cadherin staining was predictive of longer time to progression (12.4 vs. 5.9 months, $p<0.05$) and overall survival (OS) (18.4 vs. 13.0 months, $p<0.05$). Together the patients with an EGFR mutation and the patients with positive E-cadherin staining defined a patient group with a median OS of 18.4 months and excluded the patient group with the median OS of 3.7 months. Neither p-Akt nor p-EGFR staining was associated with the response and survival. In patients with surgically resected NSCLC tumors, the EGFR

mutation status and E-cadherin staining can select patients who will benefit from gefitinib therapy.

Molina-Vila, M. A., J. Bertran-Alamillo, et al. (2008). "A sensitive method for detecting EGFR mutations in non-small cell lung cancer samples with few tumor cells." J Thorac Oncol **3**(11): 1224-35.

BACKGROUND: Detection of epidermal growth factor receptor (EGFR) mutations in advanced non-small cell lung cancer (NSCLC) patients has relied on DNA purification from biopsies, amplification, and sequencing. However, the number of tumor cells in a sample is often insufficient for EGFR assessment. METHODS: We prospectively screened 1380 NSCLC patients for EGFR mutations but found that 268 were not evaluable because of insufficient tumor tissue. We therefore developed and validated a method of detecting EGFR mutations in these samples. Tumor cells were microdissected into polymerase chain reaction buffer and amplified. EGFR mutations were detected by length analysis of fluorescently labeled polymerase chain reaction products and TaqMan assay. RESULTS: We determined EGFR status in 217 (81%) of the 268 primary NSCLC samples not evaluable in our original study-fresh and paraffin-embedded with less than 150 cells. Exon 19 deletions were detected in 11.5% of patients and exon 21 L858R mutations in 5.5%. In addition, the exon 20 T790M mutation was detected in 6 of 15 (40%) patients at the time of progression to erlotinib. The primary, sensitive mutation was present in all tumor cells, whereas the T790M mutation was absent in some groups. CONCLUSIONS: The method presented here eliminates the need for DNA purification and allows for detection of EGFR mutations in samples containing as few as eight cancer cells.

Na, II, J. K. Rho, et al. (2007). "The survival outcomes of patients with resected non-small cell lung cancer differ according to EGFR mutations and the P21 expression." Lung Cancer **57**(1): 96-102.

The aims of this study were to evaluate the prognostic implications of patients with epidermal growth factor receptor (EGFR) mutations and a p21 expression, and to determine their associations in resected non-small cell lung cancer (NSCLC) patients. We sequenced exons 18-21 of the EGFR tyrosine kinase domain by performing mutation analysis of tissues from patients that suffered with NSCLC and who also had undergone surgical resection. The expressions of p21 and p53 were analyzed using immunohistochemistry. We detected EGFR mutations in 24 of 97 patients (25%). EGFR mutations were more frequent in the people who had never smoked than in the smokers (33% versus 14%, respectively; P=.028). The presence of EGFR mutations had no effect on survival. The expression of p21 in the patients with wild-type EGFR tended to be associated with better survival. However, the expression of p21 in the patients with EGFR mutations was associated with poor overall survival (P=.006). The five-year survival rates were 17% for the patients with EGFR mutations and p21 positivity (Group I), 44% for the patients with wild type EGFR (Group II), and 75% for the patients with EGFR mutation and no p21 positivity (Group III) (P=.036). Multivariate analysis that was corrected for age, gender and cancer stage revealed different overall survival outcomes according to the three groups (P=.004). There was no significant correlation between the expressions of p21 and p53. Survival outcomes in the patients with resected NSCLC may be correlated with the presence of a p21 expression and EGFR mutations.

Nemunaitis, J., M. Nemunaitis, et al. (2009). "Phase II trial of Belagenpumatucel-L, a TGF-beta2 antisense gene modified allogeneic tumor vaccine in advanced non small cell lung cancer (NSCLC) patients." Cancer Gene Ther **16**(8): 620-4.

In a previous dose escalation trial we demonstrated dose related survival correlation to Belagenpumatucel-L. In order to further evaluate safety and response at the previously defined optimal dose and schedule and to gain preliminary evidence on a hypothesis that the level of circulating tumor cells (CTCs) in blood may correlate with the overall survival of patients with stage IV NSCLC, we initiated a phase II trial. Patients received intradermal immunization of 2.5 x 10(7) transfected allogeneic tumor cells (Belagenpumatucel-L, supplied by NovaRx) 1 x every month for a total of 16 months. Circulating tumor cells (Veridex, Raritan, NJ) were measured every 4 weeks. Twenty-one advanced NSCLC patients were enrolled on this study. No significant toxic effect was observed. Overall survival was 562 days. The median survival was 660 days in patients having less than 2 CTCs at baseline compared to 150 days in patients with 2 or more CTCs (P=0.025). Phase II results of safety and response are consistent with prior experience following treatment with Belagenpumatucel-L and there is a suggestion that the number of circulating tumor cells at baseline appears to correlate with overall survival. A larger clinical trial is warranted to further explore this observation.

Noh, E. J., E. R. Jang, et al. (2005). "Methyl CpG-binding domain protein 3 mediates cancer-selective cytotoxicity by histone deacetylase inhibitors via differential transcriptional reprogramming in lung cancer cells." Cancer Res **65**(24): 11400-10.

Histone deacetylase inhibitors (HDI) have been reported to inhibit the growth and survival of cancer cells while leaving normal cells untouched. However, the mechanisms underlying this selective cell death are poorly understood. Gene expression analysis revealed that HDI treatment induced up-regulation of p21(WAF1/Cip1) and down-regulation of ErbB2 in cancer cells but not normal cells. Overexpression of p21(WAF1/Cip1) and/or silencing of ErbB2 enhanced cancer cell growth inhibition, suggesting that HDI-induced up-regulation/down-regulation of these genes play critical roles in HDI-induced growth inhibition of cancer cells. Most importantly, we found that the gene silencing factor methyl CpG-binding domain protein 3 (MBD3) was not only released from cancer-selective promoter of the HDI up-regulated p21(WAF1/Cip1) gene but also recruited to that of the HDI-down-regulated ErbB2 gene. Furthermore, silencing of MBD3 by small interfering RNA abrogated the HDI-induced gene regulation and growth inhibition in lung cancer but not in normal cells. Together, our results support the critical potential of MBD3 in HDI-induced cancer-selective cell death via cancer differential gene expression.

Noro, R., A. Gemma, et al. (2006). "Gefitinib (IRESSA) sensitive lung cancer cell lines show phosphorylation of Akt without ligand stimulation." BMC Cancer **6**: 277.

BACKGROUND: Phase III trials evaluating the efficacy of gefitinib (IRESSA) in non-small cell lung cancer (NSCLC) lend support to the need for improved patient selection in terms of gefitinib use. Mutation of the epidermal growth factor receptor (EGFR) gene is reported to be associated with clinical responsiveness to gefitinib. However, gefitinib-sensitive and prolonged stable-disease-defined tumors without EGFR gene mutation have also been reported. METHODS: To identify other key factors involved in gefitinib sensitivity, we analyzed the protein expression of molecules within the EGFR family, PI3K-Akt and Ras/MEK/Erk pathways and examined the sensitivity to gefitinib using the MTT cell proliferation assay in 23 lung cancer cell lines. RESULTS: We identified one highly sensitive cell line (PC9), eight cell lines displaying intermediate-sensitivity, and 14 resistant cell lines. Only PC9 and PC14 (intermediate-sensitivity) displayed an EGFR gene mutation including amplification. Eight out of the nine cell lines showing sensitivity had Akt phosphorylation without ligand stimulation, while only three out of the 14 resistant lines displayed this characteristic (P = 0.0059). Furthermore, the ratio of phosphor-Akt/total Akt in sensitive cells was higher than that observed in resistant cells (P = 0.0016). Akt phosphorylation was partially inhibited by gefitinib in all sensitive cell lines. CONCLUSION: These results suggest that Akt phosphorylation without ligand stimulation may play a key signaling role in gefitinib sensitivity, especially intermediate-sensitivity. In addition, expression analyses of the EGFR family, EGFR gene mutation, and FISH (fluorescence in situ hybridization) analyses showed that the phosphorylated state of EGFR and Akt might be a useful clinical marker of Akt activation without ligand stimulation, in addition to EGFR gene mutation and amplification, particularly in adenocarcinomas.

Noro, R., A. Gemma, et al. (2007). "PTEN inactivation in lung cancer cells and the effect of its recovery on treatment with epidermal growth factor receptor tyrosine kinase inhibitors." Int J Oncol **31**(5): 1157-63.

To understand the mechanisms of PTEN inactivation, which is reported to be involved in tumor progression and drug resistance in lung cancer, we analyzed the expression levels of PTEN at mRNA and protein levels, along with the genetic and epigenetic status of the PTEN gene, in a panel of lung cancer cell lines. Western blot analysis showed that six out of 25 (24%) cell lines displayed low expression of PTEN protein. The level of PTEN mRNA correlated well with corresponding protein expression in each of these six cell lines. In two of the six cell lines genomic analysis revealed homozygous deletions of the PTEN gene. Another two of the six cell lines displayed hypermethylation of the PTEN gene promoter assessed by methylation-specific PCR. The levels of PTEN mRNA and protein expression in PC9/f9 and PC9/f14 cells, which are gefitinib-resistant derivatives of the gefitinib-sensitive cell line, PC9, were reduced compared to the parental line. After treatment with the demethylating agent 5-aza-2'deoxycytidine (5-AZA) and the histone deacetyltransferase (HDAC) inhibitor Trichostatin A (TSA), the expression levels of PTEN mRNA and protein in these four cell lines (PC9/f9, PC9/f14, PC10 and PC14) were actually restored. In summary, reduction in PTEN protein expression was regulated by histone deacetylation and hypermethylation of the gene promoter, as well as homozygous deletion. In addition, we demonstrated that the combination treatment of gefitinib and TSA induced significant growth inhibition in gefitinib-resistant PC9/f9 and PC9/f14 cells. These findings suggest that the combination of the epidermal growth factor receptor tyrosine kinase inhibitor gefitinib with the demethylating agent 5-AZA and the HDAC inhibitor TSA may be a useful strategy for the treatment of some lung cancers.

Ohira, T., S. Akutagawa, et al. (2002). "Up-regulated gene expression of angiogenesis factors in post-chemotherapeutic lung cancer tissues determined by cDNA macroarray." Oncol Rep **9**(4): 723-8.

The differential expressions of hundreds of tightly transcriptionally controlled genes in freshly isolated human lung cancers and respective normal lung tissues were analyzed by the cDNA macroarray technique. Three lung cancer patients received pre-operative chemotherapy with cisplatin containing regimens. After chemotherapy, these patients underwent surgery, and poly (A)-RNA expressions of 588 genes in the samples prepared from the lung cancer and normal lung tissues were compared. These expressions of the 588 genes were demonstrated by spotting onto a filter. Histogram analysis of gene expression revealed the tumors to show commonly up-regulated expression of angiogenesis and invasion related genes and adhesion molecules such as fibroblast growth factor 3 (FGFR3), matrix metalloproteinase (MMP)15, 16 and 10, integrin beta 4, integrin alpha 9, endonexin, and several types of collagens. Thus, post-chemotherapeutic tissues from lung cancer parents are characterized by remarkable up-regulation of molecules related to angiogenesis, invasion and adhesion. Tree view showed close clustering of angiogenesis related genes. Furthermore, when the angiogenesis related genes were selected and clustered, they were categorized into three groups depending upon gene expression profiles. These results suggest that angiogenesis related molecules are suitable candidates for target-based therapeutics and angiogenesis inhibitors are expected to be effective in lung cancer patients pretreated with chemotherapy.

Ohnishi, K., J. Yasumoto, et al. (2006). "LY294002, an inhibitor of PI-3K, enhances heat sensitivity independently of p53 status in human lung cancer cells." Int J Oncol **29**(1): 249-53.

The aim of this study was to ascertain whether LY294002, an inhibitor of PI-3K, enhances heat sensitivity in human cancer cells regardless of their p53 status. Colony formation assays showed that LY294002 enhanced heat sensitivity in two human lung cancer cell lines; H1299/wild-type p53 (wtp53) and H1299/mutated p53 (mp53) cells. These cell lines have identical genetic backgrounds except for their p53 status. LY294002 suppressed the heat-induced accumulation of heat shock protein 27 (hsp27) and heat shock protein 72 (hsp72) in these cell lines. Heat-induced apoptosis was observed more frequently in H1299/wtp53 cells than in H1299/mp53 cells, and was enhanced by LY294002 in both cell lines. In addition, both the heat-induced phosphorylation of Akt and the accumulation of survivin were suppressed by LY294002. These results suggest that LY294002 inhibits anti-apoptosis signaling through hsp27 and hsp72 as well as cell survival signaling through Akt and survivin. LY294002 appears to be an attractive candidate for a p53-independent heat sensitizer in hyperthermic cancer therapy.

Okabe, T., I. Okamoto, et al. (2007). "Differential constitutive activation of the epidermal growth factor receptor in non-small cell lung cancer cells bearing EGFR gene mutation and amplification." Cancer Res **67**(5): 2046-53.

The identification of somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) in patients with non-small cell lung cancer (NSCLC) and the association of such mutations with the clinical response to EGFR tyrosine kinase inhibitors (TKI), such as gefitinib and erlotinib, have had a substantial effect on the treatment of this disease. EGFR gene amplification has also been associated with an increased therapeutic response to EGFR-TKIs. The effects of these two types of EGFR alteration on EGFR function have remained unclear, however. We have now examined 16 NSCLC cell lines, including eight newly established lines from Japanese NSCLC patients, for the presence of EGFR mutations and amplification. Four of the six cell lines that harbor EGFR mutations were found to be positive for EGFR amplification, whereas none of the 10 cell lines negative for EGFR mutation manifested EGFR amplification, suggesting that these two types of EGFR alteration are closely associated. Endogenous EGFRs expressed in NSCLC cell lines positive for both EGFR mutation and amplification were found to be constitutively activated as a result of ligand-independent dimerization. Furthermore, the patterns of both EGFR amplification and EGFR autophosphorylation were shown to differ between cell lines harboring the two most common types of EGFR mutation (exon 19 deletion and L858R point mutation in exon 21). These results reveal distinct biochemical properties of endogenous mutant forms of EGFR expressed in NSCLC cell lines and may have implications for treatment of this condition.

Okabe, T., I. Okamoto, et al. (2008). "Synergistic antitumor effect of S-1 and the epidermal growth factor receptor inhibitor gefitinib in non-small cell lung cancer cell lines: role of gefitinib-induced down-regulation of thymidylate synthase." Mol Cancer Ther **7**(3): 599-606.

Somatic mutations in the epidermal growth factor receptor (EGFR) gene are associated with the therapeutic response to EGFR tyrosine kinase inhibitors (TKI) in patients with advanced non-small cell lung cancer (NSCLC). The response rate to these drugs remains low, however, in NSCLC patients with

wild-type EGFR alleles. Combination therapies with EGFR-TKIs and cytotoxic agents are considered a therapeutic option for patients with NSCLC expressing wild-type EGFR. We investigated the antiproliferative effect of the combination of the oral fluorouracil S-1 and the EGFR-TKI gefitinib in NSCLC cells of differing EGFR status. The combination of 5-fluorouracil and gefitinib showed a synergistic antiproliferative effect in vitro in all NSCLC cell lines tested. Combination chemotherapy with S-1 and gefitinib in vivo also had a synergistic antitumor effect on NSCLC xenografts regardless of the absence or presence of EGFR mutations. Gefitinib inhibited the expression of the transcription factor E2F-1, resulting in the down-regulation of thymidylate synthase at the mRNA and protein levels. These observations suggest that gefitinib-induced down-regulation of thymidylate synthase is responsible, at least in part, for the synergistic antitumor effect of combined treatment with S-1 and gefitinib and provide a basis for clinical evaluation of combination chemotherapy with S-1 and EGFR-TKIs in patients with solid tumors.

Okuda, K., H. Sasaki, et al. (2008). "Met gene copy number predicts the prognosis for completely resected non-small cell lung cancer." Cancer Sci **99**(11): 2280-5.

The Met oncogene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF). Uncontrolled activation of Met is oncogenic and has been implicated in the growth, invasion and metastasis in a variety of tumors. Several distinct mechanisms including amplification, translocation or mutation of Met may underlie uncontrolled Met activation. In several solid tumors, amplification and mutation of Met were reported to be associated with tumorigenesis, invasion and metastasis. The present study evaluated the amplification and mutation of Met in a large number of non-small cell lung cancer (NSCLC). Among 213 NSCLC patients, increased Met copy number was identified in 12 patients (5.6%) and associated with a worse prognosis (P = 0.0414). The mutation of Met in 534 NSCLC patients was also evaluated. In these patients there were no previously reported mutations within the juxtamembrane (JM) domain (R988C, T1010I, S1058P and G1085X). However, a somatic exon 14 deleting splice variant in 3 (1.7%) of 178 NSCLC samples was identified for which sequencing was performed. Met amplification and mutation were rare in Japanese NSCLC. However, the results support a critical role of Met gene dose in NSCLC, suggesting that Met may be a specific molecular therapeutic target in selected NSCLC patients with increased Met copy number.

Osada, H., Y. Tatematsu, et al. (2005). "Histone modification in the TGFbetaRII gene promoter and its significance for responsiveness to HDAC inhibitor in lung cancer cell lines." Mol Carcinog **44**(4): 233-41.

We previously reported silencing of the TGF-beta type II receptor gene (TGFbetaRII), involving histone deacetylation, instead of DNA methylation (DNA-Me). Because different histone modifications may play crucial roles in the epigenetic alterations, we further studied links with silencing of the TGFbetaRII gene promoter in six lung cancer cell lines. ChIP assays demonstrated three chromatin patterns for this gene silencing (Pattern I: histone H3 acetylation (H3-Ac)(+/-)/histone H3 lysine 4 methylation (H3K4-Me)(+)/DNA-Me(-), Pattern II; H3-Ac(-)/H3K4-Me(+/-)/DNA-Me(-), and Pattern III; H3-Ac(-)/H3K4-Me(-)/DNA-Me(+)), indicating possible progressive alterations with H3K4-Me alteration. With exposure to a histone deacetylase inhibitor (HDAC-I), trichostatin A, cell lines with the pattern II demonstrated strong and persistent induction of TGFbetaRII expression, while those with the pattern III showed only weak or no induction. ACC-LC-91 cell line, one of the pattern II examples demonstrated strong and continuous induction of H3K4-Me similar to TGFbetaRII expression. In contrast, ACC-LC-176 with the pattern III showed only weak and transient induction of H3K4-Me, similar to TGFbetaRII expression. Treatment with 5-aza-2'-deoxycytidine (5aza-dC) in addition to HDAC-I resulted in strong and continuous induction of TGFbetaRII expression and H3K4-Me in ACC-LC-176, although 5aza-dC alone was without such effects. In ACC-LC-91, both H3-Ac and H3K4-Me were promptly and simultaneously induced by HDAC-I, and similarly inhibited by wortmannin, a PI3K family inhibitor, together with TGFbetaRII induction. These findings suggested progressive alterations of chromatin configuration including H3K4-Me alteration in TGFbetaRII gene silencing. A possible involvement of a wortmannin-sensitive kinase in histone modification was also suggested.

Oshita, F., S. Matsukuma, et al. (2006). "Novel heteroduplex method using small cytology specimens with a remarkably high success rate for analysing EGFR gene mutations with a significant correlation to gefitinib efficacy in non-small-cell lung cancer." Br J Cancer **95**(8): 1070-5.

We conducted a feasibility study to examine whether small numbers of cancer cells could be utilised for analysis of the EGFR gene status using the loop-hybrid mobility shift assay, which is a modified heteroduplex technique. Cytology specimens obtained by transbronchial abrasion were successfully used for analysis of the EGFR gene status in 50 of 52 (96.2%)

patients diagnosed with class V non-small-cell carcinoma. Furthermore, the relationship between the EGFR gene status and clinical outcome was analysed in 25 patients treated with gefitinib. Overall, 10 of 11 patients with EGFR mutations in exon 19 or 21 showed tumour regression with gefitinib treatment, compared to only two of 14 patients with wild-type EGFR. The response rate was significantly higher in the EGFR mutation group than in the wild-type EGFR group (90.9 vs 14.3%, P=0.00014). Logistic regression analysis revealed that EGFR mutations in cytology specimens represented an independent predictor of the gefitinib response. The overall and progression-free survivals were significantly longer in the EGFR mutation group than in the wild-type EGFR group (P<0.05). In conclusion, cytology specimens could be useful for analysing the EGFR status in the majority of patients with non-small-cell lung cancer to determine whether they are likely to benefit from gefitinib treatment.

Pallis, A. G., A. Voutsina, et al. (2007). "'Classical' but not 'other' mutations of EGFR kinase domain are associated with clinical outcome in gefitinib-treated patients with non-small cell lung cancer." Br J Cancer **97**(11): 1560-6.

'Classical' mutations in the EGFR tyrosine kinase domain (exons 18, 19 and 21) have been associated with sensitivity to tyrosine kinase inhibitors (TKIs) in patients with NSCLC. The aim of the current study was to evaluate whether other than the classical G719X, DEL19 and L858R mutations of EGFR confer sensitivity to TKIs. Genomic DNA was extracted from microdissected formalin-fixed paraffin-embedded tumour tissue from 86 patients treated with gefitinib. Exons 18, 19 and 21 were amplified and subjected to direct sequencing. Eleven (13%) patients harboured the classical exon's 18, 19 and 21 mutations, while 14 (16%) had 'other' variants. There was a significantly higher percentage of 'never-smoker' patients with 'classical' EGFR mutations (P=0.002). Among patients with 'classical' mutations 3 patients achieved PR and 7 SD, while in the 'other' mutations group 10 patients had SD as best response. In the wild-type group, there were 3 patients with PR and 25 with SD. Median TTP was 16, 64 (P=0.002) and 21 weeks and median survival was 36, 78 and 67 weeks for patients with wild-type, 'classical' and 'other' EGFR mutations, respectively. The clinical relevance of 'other' EGFR mutation variants remains uncertain and requires further assessment in a prospective study.

Pao, W. (2006). "Defining clinically relevant molecular subsets of lung cancer." Cancer Chemother Pharmacol **58 Suppl 1**: s11-5.

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, induce dramatic responses in certain patients with non-small cell lung cancer (NSCLC). As such, the drugs provide an unexpected tool to dissect clinically relevant molecular subsets of NSCLC. For example, using mutational profiling of tumor DNA from patients with sensitivity, primary resistance, and secondary resistance to these agents, we and others have demonstrated that somatic mutations in the tyrosine kinase domain of EGFR are associated with sensitivity to gefitinib and erlotinib, while mutations in KRAS, which encodes a GTPase downstream of EGFR, are associated with primary resistance. Furthermore, second site mutations in the EGFR kinase domain are commonly found in patients with acquired resistance. We are now using a variety of molecular and biological approaches to help further define molecular subsets of lung cancer that have relevance in the clinic.

Pao, W. and V. A. Miller (2005). "Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions." J Clin Oncol **23**(11): 2556-68.

PURPOSE: Gefitinib and erlotinib are small molecules that selectively inhibit epidermal growth factor receptor (EGFR) tyrosine kinase activity. When these drugs were introduced into the clinic, the specific targets affected in human tumors were unknown. In April 2004, two groups reported that mutations in the tyrosine kinase domain of EGFR are strongly associated with gefitinib sensitivity in patients with non-small-cell lung cancer (NSCLC). We subsequently extended these findings and showed that such mutations are also associated with sensitivity to erlotinib. Here, we present current knowledge about EGFR mutations in the context of clinical trials involving gefitinib and erlotinib in NSCLC. DESIGN: This article reviews the rationale for targeting EGFR, the development of gefitinib and erlotinib, the discovery of EGFR mutations, and subsequent studies to define the incidence, spectrum, and functions of EGFR mutations. RESULTS: The discovery of EGFR mutations promises to alter the ways in which we consider and treat NSCLC. CONCLUSION: This information can guide practitioners and help them inform their patients about EGFR mutations and their impact on the treatment of NSCLC.

Park, K. H., S. G. Lo Han, et al. (2006). "Single nucleotide polymorphisms of the TGFB1 gene and lung cancer risk in a Korean population." Cancer Genet Cytogenet **169**(1): 39-44.

The present study was conducted to investigate the association between single nucleotide polymorphisms (SNPs) of the transforming growth factor-beta 1 (TGFB1) gene and susceptibility to lung cancer and the clinical effect of the SNPs on lung cancer progression in a Korean population. Two polymorphisms in the promoter region of the TGFB1 (T-1572C, C-509T), and one SNP in codon 10 (T+869C) were determined using a SNaPshot primer extension assay in 194 Korean lung cancer patients and 283 normal controls. The polymorphic allele frequencies of A-1572G, C-509T, and T+869C were similar among lung cancer patients (0.52, 0.47, and 0.47, respectively) and controls (0.54, 0.46, and 0.44, respectively). When the data was stratified for smoking history, patients who smoked heavily and had heterozygous C-509T and T+869C genotypes showed an increased lung cancer risk (odds ratio OR = 3.77, confidence interval 95% CI = 1.25-11.30, P = 0.017; OR = 3.61, 95% CI = 1.21-10.74, P = 0.021 for each), after adjustment for age and sex. When heterozygous and homozygous variants for each SNPs were analyzed together, patients who were smokers and had variant genotypes also showed increased risk compared to the reference group. Further analyses to test the effect of the SNPs on the clinical parameters did not reveal an association of each polymorphic allele to the tumor stage or response to treatment. In addition, DNA fragments containing polymorphic genotype of the promoter region (-509T) showed increased transcriptional activity in luciferase assays using non-small cell lung cancer cell lines. In conclusion, this study suggests that heavy smokers in this Korean population who have specific polymorphic variants, which have been associated with increased transcriptional activity of TGFB1, might be more vulnerable to lung cancer.

Pataer, A., S. A. Vorburger, et al. (2002). "Adenoviral transfer of the melanoma differentiation-associated gene 7 (mda7) induces apoptosis of lung cancer cells via up-regulation of the double-stranded RNA-dependent protein kinase (PKR)." Cancer Res **62**(8): 2239-43.

Adenoviral-mediated overexpression of the melanoma differentiation-associated gene 7 (Ad-mda7) induces apoptosis in a wide range of cancer cells, although themechanism is not well understood. We report that Ad-mda7 induces andactivates the double-stranded RNA-dependent protein kinase (PKR), which leads to phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2 (eIF-2alpha) and the induction of apoptosis in lung cancer cells. Treatment with 2-aminopurine (2-AP), a serine/threonine kinase inhibitor, inhibits PKR activation, eIF2alpha phosphorylation, and apoptosis induction by Ad-mda7. Additionally, PKR null but not wild-type fibroblasts are resistant to Ad-mda7-induced apoptosis. These results suggest that the activation of PKR and its downstream targets may be a critical pathway for Ad-mda7-mediated apoptosis.

Pugh, T. J., G. Bebb, et al. (2007). "Correlations of EGFR mutations and increases in EGFR and HER2 copy number to gefitinib response in a retrospective analysis of lung cancer patients." BMC Cancer **7**: 128.

BACKGROUND: Gefitinib, a small molecule tyrosine kinase inhibitor of the Epidermal Growth Factor Receptor (EGFR), has shown limited efficacy in the treatment of lung cancer. Recognized clinical predictors of response to this drug, specifically female, non-smoker, Asian descent, and adenocarcinoma, together suggest a genetic basis for drug response. Recent studies have addressed the relationship between response and either sequence mutations or increased copy number of specific receptor tyrosine kinases. We set out to examine the relationship between response and the molecular status of two such kinases, EGFR and HER2, in 39 patients treated with gefitinib at the BC Cancer Agency. METHODS: Archival patient material was reviewed by a pathologist and malignant cells were selectively isolated by laser microdissection or manual recovery of cells from microscope slides. Genomic DNA was extracted from 37 such patient samples and exons 18-24, coding for the tyrosine kinase domain of EGFR, were amplified by PCR and sequenced. EGFR and HER2 copy number status were also assessed using FISH in 26 samples. Correlations between molecular features and drug response were assessed using the two-sided Fisher's exact test. RESULTS: Mutations previously correlated with response were detected in five tumours, four with exon 19 deletions and one with an exon 21 missense L858R point mutation. Increased gene copy number was observed in thirteen tumours, seven with EGFR amplification, three with HER2 amplification, and three with amplification of both genes. In our study cohort, a correlation was not observed between response and EGFR mutations (exon 19 deletion p = 0.0889, we observed a single exon 21 mutation in a non-responder) or increases in EGFR or HER2 copy number (p = 0.552 and 0.437, respectively). CONCLUSION: Neither mutation of EGFR nor increased copy number of EGFR or HER2 was diagnostic of response to gefitinib in this cohort. However, validation of these features in a larger sample set is appropriate. Identification of additional predictive biomarkers beyond EGFR status may be necessary to accurately predict treatment outcome.

Qin, M., B. Escuadro, et al. (2005). "Gene transfer mediated by native versus fibroblast growth factor-retargeted adenoviral vectors into lung cancer cells." Am J Respir Cell Mol Biol **32**(3): 211-7.

We compared native Adenoviral (Ad) vectors to a basic Fibroblast Growth Factor-retargeted Adenovirus (FGF2-Ad) for gene delivery into a diverse panel of lung cancer cells in vitro and xenografts in vivo. Cells were first evaluated for vector-specific receptor expression. Marked variations of surface coxsackie-adenovirus receptor (CAR), but relatively similar levels of alpha v integrin and FGF receptor expression were evident. Transduction efficiency by Ad directly correlated (R = 0.77, 95% CI 0.28-0.94, P = 0.0085) with CAR, but not with alpha v integrin expression. Transduction efficiency by FGF2-Ad did not correlate with the measured FGF receptor expression. Blocking studies indicated that gene transfer by FGF2-Ad occurred by a CAR-independent pathway, and could be inhibited by free FGF in a dose-dependent manner. Ad-antiserum inhibited FGF2-Ad gene transfer, suggesting that the Ad-component was needed for post-entry DNA-delivery. Soluble heparin sulfate proteoglycans (HSPG) or alpha v integrin blockers marginally decreased FGF2-Ad transduction. Both Ad and FGF2-Ad equally transduced CAR-positive non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) cells. By contrast, FGF2-Ad had a distinct transduction advantage in CAR-deficient NSCLC cells. This improvement in transduction of CAR-deficient cells by FGF2-Ad persisted in vivo. These data justify the need for an improved FGF2-Ad vector for clinical use in CAR-deficient lung cancer.

Ramalingam, S., J. Forster, et al. (2008). "Dual inhibition of the epidermal growth factor receptor with cetuximab, an IgG1 monoclonal antibody, and gefitinib, a tyrosine kinase inhibitor, in patients with refractory non-small cell lung cancer (NSCLC): a phase I study." J Thorac Oncol **3**(3): 258-64.

PURPOSE: To determine the optimal doses of the antiepidermal growth factor receptor (anti-EGFR) monoclonal antibody cetuximab and the EGFR tyrosine kinase inhibitor gefitinib when administered as a combination for patients with advanced/metastatic non-small cell lung cancer (NSCLC) previously treated with platinum-based chemotherapy. PATIENTS AND METHODS: Patients with advanced/metastatic NSCLC treated with prior platinum-based chemotherapy received escalating doses of weekly cetuximab (100, 200, and 250 mg/m(2), IV) and fixed doses of gefitinib (250 mg/d, PO) until disease progression or unacceptable toxicity. Available tumor samples were analyzed for EGFR expression, EGFR gene copy number and mutations, and K-RAS mutations. RESULTS: Thirteen patients were enrolled in three cohorts. Treatment was generally well-tolerated at all doses. One grade 3 headache, observed on the first treatment cycle was initially considered dose-limiting toxicity (DLT); this event was eventually determined to be caused by a brain metastasis, not toxicity. Three cases of grade 3/4 hypomagnesemia and 1 case of grade 3 skin rash occurred in the highest-dose cohort. Grade 1/2 infusion reactions occurred in three patients without requiring treatment discontinuation. Four patients (31%) achieved stable disease, no responses were observed. None of the patients had EGFR mutations or gene amplification in their tumor samples. CONCLUSION: Dual EGFR inhibition with cetuximab and gefitinib is feasible; the combination can be safely administered and may have modest activity in advanced/metastatic NSCLC. Cetuximab 250 mg/m(2) weekly IV and gefitinib 250 mg/d PO is the recommended phase II dose, although the potential for late-onset hypomagnesemia warrants close monitoring of patients receiving this combined dosage.

Ramirez, J. L., R. Rosell, et al. (2005). "14-3-3sigma methylation in pretreatment serum circulating DNA of cisplatin-plus-gemcitabine-treated advanced non-small-cell lung cancer patients predicts survival: The Spanish Lung Cancer Group." J Clin Oncol **23**(36): 9105-12.

PURPOSE: Survival in patients with advanced non-small-cell lung cancer (NSCLC) who are treated with platinum-based chemotherapy is rather variable. Methylation-dependent transcriptional silencing of 14-3-3sigma, a major G2-M checkpoint control gene, could be a predictor of longer survival. PATIENTS AND METHODS: A sensitive methylation-specific polymerase chain reaction assay was used to evaluate 14-3-3sigma methylation status in pretreatment serum DNA obtained from 115 cisplatin-plus-gemcitabine-treated advanced NSCLC patients. RESULTS: 14-3-3sigma methylation was observed in all histologic types of 39 patients (34%). After a median follow-up of 9.8 months, median survival was significantly longer in the methylation-positive group (15.1 v 9.8 months; P = .004). Median time to progression was 8 months in the methylation-positive group and 6.3 months in the methylation-negative group (log-rank test, P = .027). A multivariate Cox regression model identified only 14-3-3sigma methylation status and Eastern Cooperative Oncology Group performance status as independent prognostic factors for survival. In an exploratory analysis, median survival for 22 methylation-positive responders has not been reached, whereas survival was 11.3 months for 29 methylation-negative

responders (P = .001). CONCLUSION Methylation of 14-3-3sigma is a new independent prognostic factor for survival in NSCLC patients receiving platinum-based chemotherapy. It can be reliably and conveniently detected in the serum, thus obviating the need for tumor tissue analysis.

Rath, P. C. and T. Mukhopadhyay (2009). "p53 gene expression and 2-methoxyestradiol treatment differentially induce nuclear factor kappa B activation in human lung cancer cells with different p53 phenotypes." DNA Cell Biol **28**(12): 615-23.

The p53 tumor suppressor gene is frequently mutated in multiple human cancers, leading to loss of wild-type p53 (wt-p53)-dependent functions and tumorigenesis. p53 gene therapy is used to induce apoptosis in human cancer cells and tumors. Activation of nuclear factor kappa B (NF-kappaB) causes resistance to both chemotherapy and apoptosis in tumor cells. We show that expression of wt-p53 from a recombinant adenovirus-p53 followed by treatment with 2-methoxyestradiol (2-ME), an endogenous, nontoxic, estrogenic metabolite, resulted in differential NF-kappaB activation and inhibitor kappaB alpha (IkappaB-alpha) degradation in three different human lung cancer cell lines with different p53 phenotypes. The H322J cells, with mutant (Arg248Gln) p53, showed NF-kappaB activation and IkappaB-alpha degradation after adeno-p53 expression + 2-ME treatment; however, these conditions separately did not activate NF-kappaB, rather caused accumulation of IkappaB-alpha. In contrast, either adeno-p53 expression or 2-ME treatment induced NF-kappaB activation in the p53-deleted H1299 cells, but H460 cells, containing wt-p53, did not show NF-kappaB activation under any of these conditions. This shows p53-dependent differential signaling to NF-kappaB by 2-ME. Since NF-kappaB activation inhibits apoptosis and causes resistance to chemotherapy, our study suggests the need to distinguish p53 phenotypes of tumors for p53 gene and 2-ME therapy.

Riely, G. J., W. Pao, et al. (2006). "Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib." Clin Cancer Res **12**(3 Pt 1): 839-44.

PURPOSE: In patients with non-small cell lung cancer (NSCLC), mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase domain have been associated with sensitivity to erlotinib and gefitinib. We undertook this study to explore the relationship between EGFR mutation type and clinical variables, including treatment with gefitinib and erlotinib. EXPERIMENTAL DESIGN: In patients with NSCLC, EGFR exon 19 deletion mutations and EGFR L858R point mutations were analyzed by nonsequencing PCR-based methods from paraffin blocks of tissue obtained before treatment. The results were correlated with clinical information (sex, pathologic subtype, race/ethnicity, treatment, and overall survival). RESULTS: The two most common EGFR mutations were identified in 24% (70 of 291; 95% confidence interval, 26%-38%) of tumors from patients with NSCLC. EGFR mutation was associated with Asian ethnicity (P = 0.0023) and being a "never smoker" (P = 0.0001). Among patients with EGFR mutations, 39% (27 of 70) had EGFR L858R, whereas 61% (43 of 70) had an EGFR exon 19 deletion. After treatment with erlotinib (n = 12) or gefitinib (n = 22), patients with EGFR mutations had a median overall survival of 20 months. After treatment with erlotinib or gefitinib, patients with EGFR exon 19 deletions had significantly longer median survival than patients with EGFR L858R (34 versus 8 months; log-rank P = 0.01). CONCLUSIONS: EGFR mutations in exons 19 or 21 are correlated with clinical factors predictive of response to gefitinib and erlotinib. Those with EGFR exon 19 deletion mutations had a longer median survival than patients with EGFR L858R point mutation. These observations warrant confirmation in a prospective study and exploration of the biological mechanisms of the differences between the two major EGFR mutations.

Rosell, R., F. Cecere, et al. (2006). "Predicting the outcome of chemotherapy for lung cancer." Curr Opin Pharmacol **6**(4): 323-31.

Lung cancer is a worldwide problem. At the time of diagnosis, 50% of patients have advanced incurable disease. Different chemotherapy combinations--with or without targeted therapies--yield similar results despite the continuous efforts of clinicians. However, molecular biological studies have already shed a great deal of light on the existence of multiple genetic aberrations that can be useful for customizing treatment. mRNA transcripts involved in DNA repair pathways, such as ERCC1 and BRCA1, confer selective resistance to cisplatin or taxanes, whereas thioredoxin confers a broad spectrum of chemoresistance. Polymorphisms in DNA repair genes and methylation of checkpoint genes in circulating serum DNA could become important predictive markers of survival in certain cisplatin-based regimens. Epidermal growth factor receptor tyrosine kinase mutations are the crux of targeted therapies, whereas epithelial-mesenchymal transitions and HER3 mRNA levels are promising ancillary markers for treatment with epidermal growth factor receptor tyrosine kinase inhibitors.

Rosell, R., M. Cuello, et al. (2006). "Treatment of non-small-cell lung cancer and pharmacogenomics: where we are and where we are going." Curr Opin Oncol **18**(2): 135-43.

PURPOSE OF REVIEW: This review highlights the numerous molecular biology findings in the field of lung cancer with potential therapeutic impact in both the near and distant future. RECENT FINDINGS: Abundant preclinical and clinical data indicate that BRCA1 mRNA expression is a differential modulator of chemotherapy sensitivity. Single nucleotide polymorphisms in the excision repair cross-complementing 1 gene (ERCC1) influence survival with cisplatin-based chemotherapy. For the first time, epidermal growth factor receptor (EGFR) mutations have been shown to predict dramatic responses in metastatic lung adenocarcinomas. The crosstalk between estrogen and EGFR pathways have also been revealed. MicroRNAs control the expression of cognate target genes and predict relapse in surgically resected non-small-cell lung cancer patients. Overexpression of the Wingless-type (Wnt) genes and methylation of Wnt antagonists have been documented in non-small-cell lung cancer. SUMMARY: Understanding the relevance of these findings can help to change the clinical practice in oncology towards customizing chemotherapy and targeted therapies, leading to improvement both in survival and in cost-effectiveness.

Rosell, R., Y. Ichinose, et al. (2005). "Mutations in the tyrosine kinase domain of the EGFR gene associated with gefitinib response in non-small-cell lung cancer." Lung Cancer **50**(1): 25-33.

The potential relevance of epidermal growth factor receptor (EGFR) mutations to non-small-cell lung cancer treatment has recently been identified. We have examined the presence of EGFR mutations in Japanese and Spanish gefitinib-treated non-small-cell lung cancer patients. A total of 34 gefitinib-treated patients were screened, 18 from Japan and 16 from Spain. Laser capture microdissection was performed for the accurate procurement of tumor cells. EGFR exons 18, 19 and 21 were amplified from genomic DNA by means of PCR, and the samples were then subjected to bi-directional automatic sequencing. EGFR somatic mutations in the tyrosine kinase domain were found in 8 of 34 patients (23.5%). Gefitinib response was observed in 7 of 8 patients (87.5%) with EGFR mutations and in 3 of 24 (12.5%) with wild-type EGFR (P=0.0003). Five deletion mutations were clustered in the region spanning codons 746 to 750 (ELREA) within exon 19. Three additional tumors had amino acid substitutions within exon 18, at codons 718 and 719. Logistic regression analysis showed that response was primarily linked to the presence of EGFR mutations and secondarily linked to female gender, non-smoker status and a greater number of prior chemotherapy regimens. The presence of EGFR mutations is a major determinant of gefitinib response, and EGFR tyrosine kinase inhibitors should be tested in clinical trials of first-line treatment of lung adenocarcinomas harboring EGFR mutations.

Rosell, R., T. Moran, et al. (2009). "Screening for epidermal growth factor receptor mutations in lung cancer." N Engl J Med **361**(10): 958-67.

BACKGROUND: Activating mutations in the epidermal growth factor receptor gene (EGFR) confer hypersensitivity to the tyrosine kinase inhibitors gefitinib and erlotinib in patients with advanced non-small-cell lung cancer. We evaluated the feasibility of large-scale screening for EGFR mutations in such patients and analyzed the association between the mutations and the outcome of erlotinib treatment. METHODS: From April 2005 through November 2008, lung cancers from 2105 patients in 129 institutions in Spain were screened for EGFR mutations. The analysis was performed in a central laboratory. Patients with tumors carrying EGFR mutations were eligible for erlotinib treatment. RESULTS: EGFR mutations were found in 350 of 2105 patients (16.6%). Mutations were more frequent in women (69.7%), in patients who had never smoked (66.6%), and in those with adenocarcinomas (80.9%) (P<0.001 for all comparisons). The mutations were deletions in exon 19 (62.2%) and L858R (37.8%). Median progression-free survival and overall survival for 217 patients who received erlotinib were 14 months and 27 months, respectively. The adjusted hazard ratios for the duration of progression-free survival were 2.94 for men (P<0.001); 1.92 for the presence of the L858R mutation, as compared with a deletion in exon 19 (P=0.02); and 1.68 for the presence of the L858R mutation in paired serum DNA, as compared with the absence of the mutation (P=0.02). The most common adverse events were mild rashes and diarrhea; grade 3 cutaneous toxic effects were recorded in 16 patients (7.4%) and grade 3 diarrhea in 8 patients (3.7%). CONCLUSIONS: Large-scale screening of patients with lung cancer for EGFR mutations is feasible and can have a role in decisions about treatment.

Rosell, R., M. Skrzypski, et al. (2007). "BRCA1: a novel prognostic factor in resected non-small-cell lung cancer." PLoS One **2**(11): e1129.

BACKGROUND: Although early-stage non-small-cell lung cancer (NSCLC) is considered a potentially curable disease following complete resection, patients have a wide spectrum of survival

according to stage (IB, II, IIIA). Within each stage, gene expression profiles can identify patients with a higher risk of recurrence. We hypothesized that altered mRNA expression in nine genes could help to predict disease outcome: excision repair cross-complementing 1 (ERCC1), myeloid zinc finger 1 (MZF1) and Twist1 (which regulate N-cadherin expression), ribonucleotide reductase subunit M1 (RRM1), thioredoxin-1 (TRX1), tyrosyl-DNA phosphodiesterase (Tdp1), nuclear factor of activated T cells (NFAT), BRCA1, and the human homolog of yeast budding uninhibited by benzimidazole (BubR1). METHODOLOGY AND PRINCIPAL FINDINGS: We performed real-time quantitative polymerase chain reaction (RT-QPCR) in frozen lung cancer tissue specimens from 126 chemonaive NSCLC patients who had undergone surgical resection and evaluated the association between gene expression levels and survival. For validation, we used paraffin-embedded specimens from 58 other NSCLC patients. A strong inter-gene correlation was observed between expression levels of all genes except NFAT. A Cox proportional hazards model indicated that along with disease stage, BRCA1 mRNA expression significantly correlated with overall survival (hazard ratio [HR], 1.98 [95% confidence interval (CI), 1.11-6]; P = 0.02). In the independent cohort of 58 patients, BRCA1 mRNA expression also significantly correlated with survival (HR, 2.4 [95%CI, 1.01-5.92]; P = 0.04). CONCLUSIONS: Overexpression of BRCA1 mRNA was strongly associated with poor survival in NSCLC patients, and the validation of this finding in an independent data set further strengthened this association. Since BRCA1 mRNA expression has previously been linked to differential sensitivity to cisplatin and antimicrotubule drugs, BRCA1 mRNA expression may provide additional information for customizing adjuvant antimicrotubule-based chemotherapy, especially in stage IB, where the role of adjuvant chemotherapy has not been clearly demonstrated.

Rundall, B. K., C. E. Denlinger, et al. (2004). "Combined histone deacetylase and NF-kappaB inhibition sensitizes non-small cell lung cancer to cell death." Surgery **136**(2): 416-25.

BACKGROUND: Non-small cell lung cancer (NSCLC) remains resistant to traditional and novel chemotherapeutic agents, relating, in part, to the activation of the antiapoptotic transcription factor NF-kappaB. We hypothesize that inhibition of NF-kappaB using BAY-11-7085 will sensitize NSCLC cells to death, induced by the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). METHODS: Five tumorigenic NSCLC cell lines (A549, H157, H358, H460, H1299) were treated with nothing, SAHA, BAY-11-7085, or both compounds. Cell death was determined by crystal violet staining. p65 nuclear translocation was determined by Western blot analysis. NF-kappaB activity was determined by reporter-gene assays and by reverse transcriptase-polymerase chain reaction of the endogenous NF-kappaB-dependent gene interleukin 8. Apoptosis was determined by DNA fragmentation. Clonogenic cell survival assays were also performed. Data was analyzed with the Student t test when appropriate. RESULTS: SAHA alone resulted in no meaningful NSCLC cell death. SAHA induced nuclear translocation of p65, which was inhibited by BAY-11-7085. SAHA significantly induced NF-kappaB-dependent transcription which was ameliorated after treatment with BAY-11-7085 (P = .01). Combined SAHA and BAY-11-7085 induced significantly more apoptosis and cell death than either drug alone (P = .002). CONCLUSIONS: Combined HDI and NF-kappaB inhibition using BAY-11-7085 sensitizes NSCLC cells to cell death and appears promising as a novel treatment strategy for this disease.

Rundall, B. K., C. E. Denlinger, et al. (2005). "Suberoylanilide hydroxamic acid combined with gemcitabine enhances apoptosis in non-small cell lung cancer." Surgery **138**(2): 360-7.

BACKGROUND: We have shown that non-small cell lung cancer (NSCLC) is resistant to the histone deacetylase inhibitor (HDI) suberoylanilide hydroxamic acid (SAHA) through upregulation of the antiapoptotic transcription factor nuclear factor-kappaB (NF-kappaB). HDIs also promote chromatin remodeling, potentially making the DNA more accessible to chemotherapy. We hypothesize that combined SAHA and gemcitabine sensitizes NSCLC to apoptosis. METHODS: Three NSCLC cell lines (A549, H358, H460) were untreated, or treated with SAHA, gemcitabine, or both agents. NF-kappaB-dependent transcription was determined by reporter gene assays, reverse transcriptase-polymerase chain reaction RT-PCR, and Western blot analysis for the NF-kappaB-regulated antiapoptotic gene MnSOD. Survival of NSCLC cells overexpressing Bfl/A1, Bcl-X(L), or MnSOD and treated with SAHA and gemcitabine was determined in the presence or absence of NF-kappaB. Survival of treated cells overexpressing HDAC-1, 2, 3 or p/CAF was determined. Apoptosis was determined by fluorescence-activated cell sorter analysis, DNA fragmentation, and caspase-3 activity. Colony formation assays were performed on cells treated concurrently and sequentially with SAHA and gemcitabine. Assays were performed in triplicate, and the Student t test was applied as appropriate. RESULTS: SAHA-activated NF-kappaB (P <or= .05)

and gemcitabine inhibited these effects (P <or= .01). Increased cell survival was observed after overexpression of antiapoptotic genes, as well as in cells overexpressing HDAC-1, -2, and -3. Fluorescence-activated cell sorter analysis, DNA fragmentation, and caspase-3 assays all showed enhanced apoptosis with combined therapy, compared with single-agent therapy (P <or= .01). Sequential treatment offered no improvement over concurrent treatment. CONCLUSIONS: Combined SAHA and gemcitabine sensitized NSCLC cells to apoptosis. Potential "proapoptotic" mechanisms for this finding include gemcitabine inhibition of SAHA-induced NF-kappaB activation and chromatin remodeling mediated by the inhibition of histone deacetylases.

Santarpia, M., G. Altavilla, et al. (2006). "From the bench to the bed: individualizing treatment in non-small-cell lung cancer." Clin Transl Oncol **8**(2): 71-6.

At the time of diagnosis, half of lung cancer patients have advanced incurable disease. Different chemotherapy combinations--with or without targeted therapies--yield similar results in spite of the continuous efforts of clinicians. However, molecular biological studies have already shed a great deal of light on the existence of multiple genetic aberrations that can be useful for customizing treatment. mRNA transcripts involved in DNA repair pathways, such as ERCC1 and BRCA1, confer selective resistance to cisplatin or taxanes. Polymorphisms in DNA repair genes and methylation of checkpoint genes in circulating serum DNA could become important predictive markers of survival to certain cisplatin-based regimens. EGFR tyrosine kinase mutations are the crux of targeted therapies.

Sasaki, H., K. Endo, et al. (2005). "EGFR Mutation status in Japanese lung cancer patients: genotyping analysis using LightCycler." Clin Cancer Res **11**(8): 2924-9.

PURPOSE: Recently, somatic mutations of the epidermal growth factor receptor (EGFR) gene were found in approximately 25% of Japanese lung cancer patients. These EGFR mutations are reported to be correlated with clinical response to gefitinib therapy. However, DNA sequencing using the PCR methods described to date is time-consuming and requires significant quantities of DNA; thus, this existing approach is not suitable for a routine pretherapeutic screening program. EXPERIMENTAL DESIGN: We have genotyped EGFR mutation status in Japanese lung cancer patients, including 102 surgically treated lung cancer cases from Nagoya City University Hospital and 16 gefitinib-treated lung cancer cases from Kinki-chuo Chest Medical Center. The presence or absence of three common EGFR mutations were analyzed by real-time quantitative PCR with mutation-specific sensor and anchor probes. RESULTS: In exon 21, EGFR mutations (CTG --> CGG; L858R) were found from 8 of 102 patients from Nagoya and 1 of 16 from Kinki. We also detected the deletion mutations in exon 19 from 7 of 102 patients from Nagoya (all were deletion type 1a) and 4 of 16 patients from Kinki (one was type 1a and three were type 1b). In exon 18, one example of G719S mutation was found from both Nagoya and Kinki. The L858R mutation was significantly correlated with gender (women versus men, P < 0.0001), Brinkman index (600 < or = versus 600, P = 0.001), pathologic subtypes (adenocarcinoma versus nonadenocarcinoma, P = 0.007), and differentiation status of the lung cancers (well versus moderately or poorly, P = 0.0439), whereas the deletion mutants were not. EGFR gene status, including the type of EGFR somatic mutation, was correlated with sensitivity to gefitinib therapy. For example, some of our gefitinib-responsive patients had L858R or deletion type 1a mutations. On the other hand, one of our gefitinib-resistant patients had a G719S mutation. CONCLUSIONS: Using the LightCycler PCR assay, the EGFR L858R mutation status might correlate with gender, pathologic subtypes, and gefitinib sensitivity of lung cancers. However, further genotyping studies are needed to confirm the mechanisms of EGFR mutations for the sensitivity or resistance of gefitinib therapy for the lung cancer.

Sasaki, H., O. Kawano, et al. (2007). "EGFRvIII mutation in lung cancer correlates with increased EGFR copy number." Oncol Rep **17**(2): 319-23.

Overexpression of the epidermal growth factor receptor (EGFR) is caused by EGFR gene amplification and is sometimes associated with expression of a variant EGFR (deletion exon 2-7 or EGFRvIII). EGFRvIII mutation has oncogenic potential and is investigated as a potential therapeutic target. We genotyped the EGFRvIII mutation status in 252 surgically treated lung cancer cases. The presence or absence of EGFRvIII mutation was analyzed by real-time quantitative polymerase chain reaction (PCR) with mutation specific sensor and anchor probes. EGFR copy number was evaluated with PCR-based assay. EGFR mutation status at kinase domain has been examined and reported. EGFRvIII mutation was found on 8 of 252 patients. All patients were male, smokers, and 7 had squamous cell carcinoma. The mutation status was significantly correlated with pathological subtypes (squamous cell carcinoma vs. adenocarcinoma, p=0.0114). Sixty EGFR mutations at kinase domain exclusively existed with EGFRvIII mutations. EGFR gene copy number was significantly higher in EGFRvIII mutant (4.711+/-4.968) than in

non-EGFRvIII mutant (2.284+/-1.224) (p=0.0001). EGFRvIII gene mutation might be one of the mechanisms of increased EGFR copy number. Further studies are needed to confirm the mechanisms of EGFRvIII mutations for possible anti-EGFR therapy for lung cancer.

Sasaki, H., K. Okuda, et al. (2009). "EGFR R497K polymorphism is a favorable prognostic factor for advanced lung cancer." J Cancer Res Clin Oncol **135**(2): 313-8.

INTRODUCTION: It has been reported that the R497K polymorphism of the epidermal growth factor receptor (EGFR) gene has attenuated functions in ligand binding, tyrosine kinase activation, and growth stimulation. On other hand, EGFR gene mutations at kinase domain in non-small cell lung cancer (NSCLC) have been examined for their ability to predict sensitivity to gefitinib or erlotinib. MATERIALS AND METHODS: We investigated the EGFR mutations and/or R497K polymorphism statuses in 225 surgically treated NSCLC cases. 192 adenocarcinoma cases were included. The presence or absence of EGFR polymorphism of exon 13 was analyzed by PCR-RFLP method. RESULTS: EGFR mutations at kinase domain were found from 95 of 225 lung cancer patients. In 86.2% of patients, homo- or heterozygous Lys497 allele was present. No correlation existed between R497K EGFR genotype and clinico-pathological features, such as gender, smoking status, and pathological subtypes. CONCLUSIONS: EGFR mutation status was not correlated with R497KEGFR genotype of lung cancers. In node-negative patients, R497KEGFR genotype was not correlated with disease outcome. In node-positive patients, however, R497K EGFR was significantly associated with better overall survival. This association was attributable to neo-adjuvant or adjuvant chemotherapy. In 46 total gefitinib treated NSCLC patients, the prognosis was not different between the EGFR wild type (GG) patients and AG+AA patients. R497KEGFR polymorphism might be associated with favorable prognosis of advanced lung cancers and correlated with chemosensitivity.

Sasaki, H., S. Shimizu, et al. (2006). "EGFR and erbB2 mutation status in Japanese lung cancer patients." Int J Cancer **118**(1): 180-4.

Much evidence has accumulated that the epidermal growth factor receptor (EGFR) and its family members are strongly implicated in the development and progression of lung cancers. Somatic mutations of the EGFR gene were found in about 25-40% of Japanese lung cancer patients. More recently, erbB2 mutations are found in about 4% of European-derived lung cancer patients. We have investigated EGFR and erbB2 mutation status in 95 surgically treated nonsmall cell lung cancer (NSCLC) cases from Nagoya City University Hospital. Seventy-five adenocarcinoma cases were included. The presence or absence of EGFR and ernB2 mutations of kinase domains were analyzed by reverse transcription polymerase chain reaction (RT-PCR) amplifications and direct sequences. We have also investigated erbB2 mutation status in 27 surgically treated NSCLC cases followed by treatment with gefitinib from Kinki-chuo Chest Medical Center. EGFR mutations (CTG-->CGG; L858R) were found from 14 of 95 lung cancer patients. We also detected the deletion 1a-type mutations from 9 patients and deletion 4-type mutations from 6 patients in exon 19. In exon 20, 4 mutations including 2 novel mutations were found. Total EGFR mutations were present in 35 patients (36.8%). These mutation statuses were significantly correlated with gender (women 73.3% vs. men 20%, $p < 0.0001$), smoking status (never smoker 69.4% vs. smoker 16.9%, $p < 0.0001$), pathologic subtypes (adenocarcinoma 45.1% vs. nonadenocarcinoma 12.5%, $p = 0.0089$) and differentiation status of the lung cancers (well 51% vs. moderately or poorly 18.4%, $p = 0.0021$). On the other hand, erbB2 mutation was only found from 1 of 95 patients, at exon 20. This patient was female and a never smoker with adenocarcinoma. This 12 nucleotide insertion mutation (2324-2325 ins ATACGTGATGGC) was located in the exon 20 at kinase domain (775-776 ins YVMA). There was no erbB2 mutation in 27 gefitinib-treated NSCLC patients. In total, we have found only 1 erbB2 mutation from 122 (0.8%) Japanese NSCLC patients. There was a significantly higher erbB2 positive (2+/3+) ratio in EGFR mutant patients (13/25, 52.0%) compared to EGFR wild-type patients (10/62, 16.1%; $p = 0.0247$). The NSCLC specimen with erbB2 mutation showed 1+ immunoreactivity. The EGFR mutation status might correlate with the clinicopathologic features related to good response to gefitinib, such as gender, smoking history and pathologic subtypes of lung cancers. However, erbB2 mutation is rare from Japanese lung cancer and is of limited value for molecular target therapy.

Sato, K., Y. Tomizawa, et al. (2006). "Epigenetic inactivation of the RUNX3 gene in lung cancer." Oncol Rep **15**(1): 129-35.

The silencing of tumor suppressor genes (TSGs) by aberrant hypermethylation occurs frequently in human cancer. Recently the RUNX3 gene was identified as a TSG inactivated by hypermethylation. We examined RUNX3 expression by reverse transcription-PCR and the methylation status of this gene by methylation specific-PCR in 43

lung cancer cell lines and 120 primary non-small cell lung cancer (NSCLC) tumor samples. RUNX3 expression was absent in 10 (50%) of 20 small cell lung cancer (SCLC) cell lines, 8 (50%) of 16 adenocarcinoma (AdC) cell lines, and 1 (33.3%) of 3 squamous cell carcinoma (SqC) cell lines. The frequency of RUNX3 methylation was significantly higher in AdC (7/16, 43.8%) than SCLC cell lines (1/20, 5%; p=0.032). RUNX3 expression was restored by treatment with 5-aza-2'-deoxycytidine and/or trichostatin-A in AdC cell lines. These results indicated that RUNX3 expression was regulated by aberrant hypermethylation in AdC cell lines. RUNX3 methylation was detected in 30 (25%) of 120 primary NSCLC tumors. RUNX3 methylation was significantly more frequent in non-smokers (16/43, 37.2%) than smokers (12/71, 16.9%; p=0.014), and in patients with AdC (26/72, 36.1%) than in patients with SqC (3/45, 6.7%; p<0.001). These results indicated that silencing of the RUNX3 gene plays an important role in the pathogenesis of lung cancer, and aberrant methylation is an important mechanism of inactivation of the RUNX3 gene in lung AdC.

Sato, M., D. S. Shames, et al. (2007). "A translational view of the molecular pathogenesis of lung cancer." J Thorac Oncol **2**(4): 327-43.

Molecular genetic studies of lung cancer have revealed that clinically evident lung cancers have multiple genetic and epigenetic abnormalities, including DNA sequence alterations, copy number changes, and aberrant promoter hypermethylation. Together, these abnormalities result in the activation of oncogenes and inactivation of tumor-suppressor genes. In many cases these abnormalities can be found in premalignant lesions and in histologically normal lung bronchial epithelial cells. Findings suggest that lung cancer develops through a stepwise process from normal lung epithelial cells towards frank malignancy, which usually occurs as a result of cigarette smoking. Lung cancer has a high morbidity because it is difficult to detect early and is frequently resistant to available chemotherapy and radiotherapy. New, rationally designed early detection, chemoprevention, and therapeutic strategies based on the growing understanding of the molecular changes important to lung cancer are under investigation. For example, methylated tumor DNA sequences in sputum or blood are being investigated for early detection screening, and new treatments that specifically target molecules such as vascular endothelial growth factor and the epidermal growth factor receptor are becoming available. Meanwhile, global gene expression signatures from individual tumors are showing potential as prognostic and therapeutic indicators, such that molecular typing of individual tumors for therapy selection is not far away. Finally, the recent development of a model system of immortalized human bronchial epithelial cells, along with a paradigm shift in the conception of cancer stem cells, promises to improve the situation for patients with lung cancer. These advances highlight the translation of molecular discoveries on lung cancer pathogenesis from the laboratory to the clinic.

Schettino, C., M. A. Bareschino, et al. (2008). "The potential role of pharmacogenomic and genomic in the adjuvant treatment of early stage non small cell lung cancer." Curr Genomics **9**(4): 252-62.

Although notable progress has been made in the treatment of non-small-cell lung cancer (NSCLC) in recent years, this disease is still associated with a poor prognosis. Despite early-stage NSCLC is considered a potentially curable disease following complete resection, the majority of patients relapse and eventually die after surgery. Adjuvant chemotherapy prolongs survival, altough the absolute improvement in 5-year overall survival is only approximately 5%.Trying to understand the role of genes which could affect drug activity and response to treatment is a major challenge for establishing an individualised chemotherapy according to the specific genetic profile of each patient. Among genes involved in the DNA repair system, the excision repair cross-complementing 1 (ERCC1) is a useful markers of clinical resistance to platinum-based chemotherapy. In the International Lung Cancer Trial (IALT) adjuvant chemotherapy significantly prolonged survival among patients with ERCC1 negative tumors but not among ERCC1-positive patients. BRCA1 and ribonucleotide reductase M1 (RRM1), two other key enzymes in DNA synthesis and repair, appear to be modulators of drug sensitivity and may provide additional information for customizing adjuvant chemotherapy.Several clinical trials suggest that overexpression of class III beta-tubulin is an adverse prognostic factor in cancer since it could be responsible for resistance to anti-tubulin agents. A retrospective analysis of NCIC JBR.10 trial showed that high tubulin III expression is associated with a higher risk of relapse following surgery alone but also with a higher probability of benefit from adjuvant cisplatin plus vinorelbine chemotherapy.Finally, the use of gene expression patterns such as the lung metagene model could provide a potential mechanism to refine the estimation of a patient's risk of disease recurrence and could affect treatment decision in the management of early stage of NSCLC.In this review we will discuss the potential role of pharmacogenomic approaches to guide the medical treatment of early stage NSCLC.

Schittenhelm, M. M., C. Kollmannsberger, et al. (2009). "Molecular determinants of response to matuzumab in combination with paclitaxel for patients with advanced non-small cell lung cancer." Mol Cancer Ther **8**(3): 481-9.

Antibodies targeting epidermal growth factor receptor (EGFR) have proven to be effective in patients with non-small cell lung cancer (NSCLC) that express EGFR. We recently published a phase I study of weekly matuzumab plus paclitaxel. This therapy was well tolerated and showed clinical responses in the majority of patients. Although matuzumab displays potent antitumor activity in some patients, not all patients respond well to treatment. Whether dysregulation of EGFR-mediated pathways precludes or sensitizes cells to paclitaxel is unknown. We sought to determine molecular predictive factors for therapy response in a phase I/II study patient cohort treated with matuzumab+/-paclitaxel. Twenty-three cases [including one complete response (CR), three partial responses (PR), 10 stable diseases (SD)] were screened using immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), PCR/sequencing and denaturing wave high performance liquid chromatography (D-HPLC) for expression, amplification, and mutation status of EGFR and downstream signaling pathways. All patients with PR or CR displayed an either high overall or single-cell EGFR expression in the majority of cells. In addition, all of the moderate responders, who achieved SD after at least two cycles of therapy, showed diffuse EGFR expression rates and/or strong single-cell EGFR expression. In contrast, 44% of the nonresponders showed low overall or single-cell EGFR expression levels. No low-expressing EGFR cases were present within the responder group. In addition, among patients with a gain-of-function mutation in KRAS primary therapy failure and/or short responses to therapy were observed. Our data suggest that EGFR expression and KRAS mutation status is predictive for clinical response to matuzumab +/- paclitaxel in patients with advanced NSCLC.

Schneider, C. P., D. Heigener, et al. (2008). "Epidermal growth factor receptor-related tumor markers and clinical outcomes with erlotinib in non-small cell lung cancer: an analysis of patients from german centers in the TRUST study." J Thorac Oncol **3**(12): 1446-53.

INTRODUCTION: Relationships between clinical outcomes and epidermal growth factor receptor (EGFR)-related tumor markers were investigated in patients with advanced non-small cell lung cancer. METHODS: Patients with stage IIIB/IV non-small cell lung cancer (0-2 prior regimens) received erlotinib (150 mg PO per day). Response and survival were evaluated, and tumor samples were assessed by immunohistochemistry (EGFR, phosphorylated mitogen-activated protein kinase, and phosphorylated AKT protein expression), fluorescence in situ hybridization (FISH; EGFR gene copy number), and DNA sequencing (EGFR, KRAS gene mutations). RESULTS: Among 311 patients, 8% had a complete/partial response; the disease control rate was 66%. Median Overall survival (OS) was 6.1 months; 1-year survival rate was 27.2%. Two of 4 patients with EGFR mutations had tumor responses, versus 2/68 with wild-type EGFR ($p = 0.014$). Progression-free survival (PFS) (HR = 0.31) and OS (HR = 0.33) were significantly prolonged in patients with EGFR mutations. Response rate was significantly higher in patients with EGFR FISH-positive (17%) than FISH-negative tumors (6%), and both PFS (HR = 0.58) and OS (HR = 0.63) significantly favored patients with EGFR FISH-positive tumors; median OS was 8.6 months in the EGFR FISH-positive group. None of 17 patients with a KRAS mutation had a tumor response, but the impact of KRAS mutation status on survival outcomes was of borderline statistical significance. Neither phosphorylated mitogen-activated protein kinase nor phosphorylated AKT immunohistochemistry status had a significant effect on PFS and OS with erlotinib. CONCLUSIONS: The presence of EGFR mutations and EGFR FISH-positive tumors may predispose patients to achieving better outcomes on erlotinib, but may have a beneficial impact on prognosis (irrespective of treatment). Prospective, placebo-controlled studies are needed to determine the predictive value of the putative biomarkers.

Seo, H. S., D. D. Liu, et al. (2008). "Cyclic AMP response element-binding protein overexpression: a feature associated with negative prognosis in never smokers with non-small cell lung cancer." Cancer Res **68**(15): 6065-73.

Lung cancer is the leading cause of cancer deaths worldwide. Recent advances in targeted therapies hold promise for the development of new treatments for certain subsets of cancer patients by targeting specific signaling molecule. Based on the identification of the transcription factor cyclic AMP response element-binding protein (CREB) as an important regulator of growth of several types of cancers and our recent findings of its importance in normal differentiation of bronchial epithelial cells, we hypothesized that CREB plays an important pathobiologic role in lung carcinogenesis. We conducted this initial study to determine whether the expression and activation status of CREB are altered in non-small cell lung cancer (NSCLC) and of any prognostic importance in NSCLC patients. We found

that the expression levels of mRNA and protein of CREB and phosphorylated CREB (p-CREB) were significantly higher in most of the NSCLC cell lines and tumor specimens than in the normal human tracheobronchial epithelial cells and adjacent normal lung tissue, respectively. Analysis of CREB mRNA expression and the CREB gene copy number showed that CREB overexpression occurred mainly at the transcriptional level. Immunohistochemical analysis of tissue microarray slides containing sections of NSCLC specimens obtained from 310 patients showed that a decreased survival duration was significantly associated with overexpression of CREB or p-CREB in never smokers but not in current or former smokers with NSCLC. These are the first reported results illustrating the potential of CREB as a molecular target for the prevention and treatment of NSCLC, especially in never smokers.

Sequist, L. V., V. A. Joshi, et al. (2006). "Epidermal growth factor receptor mutation testing in the care of lung cancer patients." Clin Cancer Res **12**(14 Pt 2): 4403s-4408s.

As the literature about epidermal growth factor receptor (EGFR) mutations grows and screening for mutations becomes increasingly integrated into clinical care, it is important to examine how best to do somatic mutational analyses and how best to use the test results in clinical decision making. We began offering mutation screening by comprehensive direct sequence analysis of exons 18 to 24 of the tyrosine kinase domain of EGFR in August 2004 as part of clinical cancer care and protocol therapy at our institutions. All identified potential mutations are confirmed with three to five independent PCRs of the original genomic DNA sample and, if not previously noted in the literature, are compared with the patient's germ-line DNA to ensure the finding is somatic. We formally analyzed the first 100 patients to undergo EGFR sequence analysis and found that testing was feasible and significantly affected the treatment of patients with non-small cell lung cancer (NSCLC). Patients harboring EGFR mutations were significantly more likely to receive recommendations for therapy with EGFR tyrosine kinase inhibitors (i.e., gefitinib or erlotinib) than patients without mutations. However, negative EGFR test results did not prevent physicians from administering these agents to selected patients. Ideally, a standardized technique for mutation testing could be developed, with demonstrated reproducibility and validity. Clinical trials incorporating molecular diagnostics are ongoing to assess the efficacy of EGFR tyrosine kinase inhibitors as first-line therapy for metastatic NSCLC and as adjuvant therapy for early-stage resected NSCLC. It is likely that mutation testing and other molecular analyses will be most useful in these two clinical situations.

Sequist, L. V., V. A. Joshi, et al. (2007). "Response to treatment and survival of patients with non-small cell lung cancer undergoing somatic EGFR mutation testing." Oncologist **12**(1): 90-8.

Somatic mutations in the epidermal growth factor receptor (EGFR) gene are associated with clinical response and prolonged survival in patients with non-small cell lung cancer (NSCLC) treated with EGFR tyrosine kinase inhibitors (TKIs). We began screening patients for somatic EGFR mutations by DNA sequencing as part of clinical care in 2004. We performed a retrospective cohort study of 278 patients with NSCLC referred for EGFR testing over a 10-month period. Tumor samples underwent direct DNA sequence analyses of EGFR exons 18 through 24. We determined the clinical characteristics and EGFR mutation status of the patients and analyzed their response to therapy and survival. EGFR somatic mutations were identified in 68 (24%) of patients. A minimal smoking history was the strongest clinical predictor of harboring a mutation. In multivariable analyses, each pack-year of smoking corresponded to a 5% decreased likelihood of having an EGFR mutation. Among 92 patients with unresectable disease undergoing subsequent systemic therapy, EGFR mutations were associated with an increased response rate to EGFR TKIs ($p < .0001$) but not chemotherapy. Overall survival was significantly prolonged in EGFR mutation-positive patients ($p = .001$), with a median survival of 3.1 years compared with 1.6 years in mutation-negative patients, after adjusting for age, gender, and stage at diagnosis. Integrating molecular profiling into clinical care is feasible in NSCLC patients and provides useful clinical information.

Shaw, A. T., B. Y. Yeap, et al. (2009). "Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK." J Clin Oncol **27**(26): 4247-53.

PURPOSE: The EML4-ALK fusion oncogene represents a novel molecular target in a small subset of non-small-cell lung cancers (NSCLC). To aid in identification and treatment of these patients, we examined the clinical characteristics and treatment outcomes of patients who had NSCLC with and without EML4-ALK. PATIENTS AND METHODS: Patients with NSCLC were selected for genetic screening on the basis of two or more of the following characteristics: female sex, Asian ethnicity, never/light smoking history, and adenocarcinoma histology. EML4-ALK was identified by using fluorescent in situ hybridization for ALK

rearrangements and was confirmed by immunohistochemistry for ALK expression. EGFR and KRAS mutations were determined by DNA sequencing. RESULTS: Of 141 tumors screened, 19 (13%) were EML4-ALK mutant, 31 (22%) were EGFR mutant, and 91 (65%) were wild type (WT/WT) for both ALK and EGFR. Compared with the EGFR mutant and WT/WT cohorts, patients with EML4-ALK mutant tumors were significantly younger (P < .001 and P = .005) and were more likely to be men (P = .036 and P = .039). Patients with EML4-ALK-positive tumors, like patients who harbored EGFR mutations, also were more likely to be never/light smokers compared with patients in the WT/WT cohort (P < .001). Eighteen of the 19 EML4-ALK tumors were adenocarcinomas, predominantly the signet ring cell subtype. Among patients with metastatic disease, EML4-ALK positivity was associated with resistance to EGFR tyrosine kinase inhibitors (TKIs). Patients in the EML4-ALK cohort and the WT/WT cohort showed similar response rates to platinum-based combination chemotherapy and no difference in overall survival. CONCLUSION: EML4-ALK defines a molecular subset of NSCLC with distinct clinical characteristics. Patients who harbor this mutation do not benefit from EGFR TKIs and should be directed to trials of ALK-targeted agents.

Shepherd, F. A. and R. Rosell (2007). "Weighing tumor biology in treatment decisions for patients with non-small cell lung cancer." J Thorac Oncol **2 Suppl 2**: S68-76.

Tumor molecular biology is an increasingly important consideration when choosing therapy for patients with advanced non-small cell lung cancer (NSCLC). A number of potential biological markers are under active investigation in the hope that it will be possible to identify markers that assist in patient selection for specific therapies. Distinguishing prognostic from predictive makers is crucial to the development of customized drug therapy. Some markers, such as mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR), are prognostic; patients with EGFR-mutant NSCLC have prolonged survival compared with those with wild-type disease, regardless of the treatment received. Although EGFR mutations are predictive of response to EGFR tyrosine kinase inhibitor (TKI) therapy, they do not appear to be predictive of a differential effect on survival. Other EGFR markers, such as protein expression or gene amplification, may be better predictors of a survival benefit from EGFR TKI. HER2 expression status and K-ras mutations provide additional information that may be useful in evaluating a patient for EGFR TKI therapy. Biological markers for chemosensitivity and resistance are also emerging. Patients with an elevated DNA repair capacity, evidenced by increased tumor expression of excision repair cross-complementing 1 or ribonucleotide reductase subunit M1 messenger RNA, may benefit less from cisplatin and gemcitabine, respectively, than from other agents. Increased levels of class III beta-tubulin are associated with taxane-resistance, and K-ras mutations have been associated with a lack of survival benefit from adjuvant chemotherapy in early stage NSCLC. It is likely that in the future, clinicians will evaluate a panel of biological markers in order to customize therapy for individual patients with NSCLC.

Shih, J. Y., C. H. Gow, et al. (2006). "Epidermal growth factor receptor mutations in needle biopsy/aspiration samples predict response to gefitinib therapy and survival of patients with advanced nonsmall cell lung cancer." Int J Cancer **118**(4): 963-9.

Recently, mutations in the epidermal growth factor receptor (EGFR) gene in nonsmall cell lung cancer (NSCLC) patients were reported to correlate with gefitinib response. Less than 30% of NSCLC patients are surgically resectable; however, molecular analysis has to rely on nonsurgical diagnostic tissue samples. The objective of this study is to investigate EGFR mutation analysis on needle biopsy/aspiration samples and its correlations with gefitinib response and patients' survival. EGFR mutation was assessed from DNA of 63 paraffin-embedded small needle biopsy/aspiration specimens from 62 patients with NSCLC treated with gefitinib. The peripheral blood lymphocyte DNA of the patients was sequenced to verify the EGFR mutation. EGFR mutations were found in 47% of 62 patients (60% of 20 CT-guided biopsies, 44% of 18 ultrasound-guided biopsies, 31% of 16 endoscopic biopsies and 44% of 9 effusion cell blocks). EGFR mutations were frequently present in females (p = 0.006) and never smokers (p = 0.04). Patients with EGFR mutations had a significantly better response rate compared to that of the nonmutation group (p < 0.001). Multivariate analysis showed that EGFR mutation (p < 0.001) and PS 0-1 (p = 0.02) were independently associated with a better response rate. Cox regression analysis showed that EGFR mutation was the independent prognostic factor for progression-free survival (p = 0.008) and overall survival (p = 0.03). In conclusion, EGFR mutation analysis is feasible in needle biopsy/aspiration paraffin-fixed specimens. EGFR mutation is an independent predictor of gefitinib response and survival in patients of advanced NSCLC treated by gefitinib.

Stuschke, M., A. Sak, et al. (2002). "Radiation-induced apoptosis in human non-small-cell lung cancer cell lines is secondary to cell-cycle progression beyond the G2-phase checkpoint." Int J Radiat Biol **78**(9): 807-19.

PURPOSE: To characterize the relationship between cell-cycle progression and radiation-induced apoptosis in NSCLC cell lines with different p53 status. MATERIALS AND METHODS: Cell lines with functional (H460, A549) and non-functional p53 (H661 and H520) were irradiated with 20 Gy. Multiparameter flow-cytometry was used to follow the progression of synchronized cells through the cell cycle after irradiation. RESULTS: Delayed apoptosis was observed after cell-cycle progression beyond the G2 block, either in the late G2/M-phase of the same cell cycle being irradiated (H661, H520) or in the G1-phase of the subsequent cell cycle (H460, A549). The apoptotic fraction in H661 and H520 was 60-80% at 144h after irradiation, higher than in A549 and H460 (5 and 35%, respectively). As an alternative to apoptosis in cells cycling beyond the G2 restriction point, hyperploid cells were generated by all cell lines. Inhibition of cell-cycle progression through the G2/M-phase efficiently reduced the induction of late apoptosis. After irradiation in S-phase, 50-60% of cells with functional p53 remained arrested at the G2 restriction point until 144 h post-irradiation, while only 20% of the H661 or H520 did so. CONCLUSIONS: These data characterize radiation-induced apoptosis in NSCLC cell lines as a removal pathway of clonogenically inactivated cells secondary to cell-cycle progression beyond G2/M, and is unlikely to be a critical factor for cellular radiation sensitivity.

Su, L., J. Zhang, et al. (2005). "Differential expression of CXCR4 is associated with the metastatic potential of human non-small cell lung cancer cells." Clin Cancer Res **11**(23): 8273-80.

PURPOSE: To evaluate the relation between CXCR4 expression and the presence of metastatic disease in human non-small cell lung cancer (NSCLC) patients and investigate whether modulation of CXCR4 expression could serve as a potential pathway in preventing metastasis of NSCLC. EXPERIMENTAL DESIGN: CXCR4 expression in 36 patients with NSCLC and 10 normal lung tissues was detected by real-time PCR and immunohistochemistry. CXCR4 expression in two human NSCLC clones (95C and 95D) with different metastatic potential was determined by real-time PCR and flow cytometry. 95C and 95D cells were transfected with the plasmid DNA containing CXCR4 coding gene or CXCR4 antisense nucleotide fragment, respectively, and the effects on in vitro cell migration, invasion, and adhesion and in vivo metastasis were measured. RESULTS: Up-regulated expression of CXCR4 was detected in 34 tumors, which were further divided into 17 high expression cancers and 17 low expression cancers by their staining intensities. High CXCR4 tumors (13 of 17) were more prone to clinical metastasis in comparison with low expression tumors. CXCR4 was differentially expressed in 95C and 95D cells with low or high metastatic potential, and the surface expression of CXCR4 were 50% up-regulated or down-regulated following the stable transfection. The metastatic potential of NSCLC in vitro, such as migration, invasion, and adhesion, were significantly enhanced or impaired. In addition, neutralizing the interactions of stromal cell-derived factor-1/CXCR4 in vitro with CXCR4-specific antibodies inhibited the CXCR4-dependent migration, invasion, and adhesion. Furthermore, s.c. inoculation of lung cancer cells with low expression of CXCR4 in nude mice showed 0- to 2-fold decrease in lung metastatic foci than that with high expression of CXCR4. CONCLUSIONS: Differential expression of CXCR4 is associated with the metastatic potential of human NSCLC, raising the possibility that blockade of CXCR4/stromal cell-derived factor-1 interaction may lead the way to design novel therapeutic tools for the treatment of metastatic NSCLC patients.

Subramanian, J. and R. Govindan (2007). "Lung cancer in never smokers: a review." J Clin Oncol **25**(5): 561-70.

Lung cancer is the leading cause of cancer-related death in the United States. Although tobacco smoking accounts for the majority of lung cancer, approximately 10% of patients with lung cancer in the United States are lifelong never smokers. Lung cancer in the never smokers (LCINS) affects women disproportionately more often than men. Only limited data are available on the etiopathogenesis, molecular abnormalities, and prognosis of LCINS. Several etiologic factors have been proposed for the development of LCINS, including exposure to radon, cooking fumes, asbestos, heavy metals, and environmental tobacco smoke, human papillomavirus infection, and inherited genetic susceptibility. However, the relative significance of these individual factors among different ethnic populations in the development of LCINS has not been well-characterized. Adenocarcinoma is the predominant histologic subtype reported with LCINS. Striking differences in response rates and outcomes are seen when patients with advanced non-small-cell lung cancer (NSCLC) who are lifelong never smokers are treated with epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitors such as gefitinib or erlotinib compared with the outcomes with these

agents in patients with tobacco-associated lung cancer. Interestingly, the activating mutations in the EGFR-TK inhibitors have been reported significantly more frequently in LCINS than in patients with tobacco-related NSCLC. This review will summarize available data on the epidemiology, risk factors, molecular genetics, management options, and outcomes of LCINS.

Suganuma, M., M. Kurusu, et al. (2006). "Green tea polyphenol stimulates cancer preventive effects of celecoxib in human lung cancer cells by upregulation of GADD153 gene." Int J Cancer **119**(1): 33-40.

To more clearly understand the molecular mechanisms involved in synergistic enhancement of cancer preventive activity with the green tea polyphenol (-)-epigallocatechin gallate (EGCG), we examined the effects of cotreatment with EGCG plus celecoxib, a cyclooxygenase-2 selective inhibitor. We specifically looked for induction of apoptosis and expression of apoptosis related genes, with emphasis on growth arrest and DNA damage-inducible 153 (GADD153) gene, in human lung cancer cell line PC-9: Cotreatment with EGCG plus celecoxib strongly induced the expression of both GADD153 mRNA level and protein in PC-9 cells, while neither EGCG nor celecoxib alone did. However, cotreatment did not induce expression of other apoptosis related genes, p21(WAF1) and GADD45. Judging by upregulation of GADD153, only cotreatment with EGCG plus celecoxib synergistically induced apoptosis of PC-9 cells. Synergistic effects with the combination were also observed in 2 other lung cancer cell lines, A549 and ChaGo K-1. Furthermore, EGCG did not enhance GADD153 gene expression or apoptosis induction in PC-9 cells in combination with N-(4-hydroxyphenyl)retinamide or with aspirin. Thus, upregulation of GADD153 is closely correlated with synergistic enhancement of apoptosis with EGCG. Cotreatment also activated the mitogen-activated protein kinases (MAPKs), such as ERK1/2 and p38 MAPK: Preteatment with PD98059 (ERK1/2 inhibitor) and UO126 (selective MEK inhibitor) abrogated both upregulation of GADD153 and synergistic induction of apoptosis of PC-9 cells, while SB203580 (p38 MAPK inhibitor) did not do so, indicating that GADD153 expression was mediated through the ERK signaling pathway. These findings indicate that high upregulation of GADD153 is a key requirement for cancer prevention in combination with EGCG.

Sunaga, N., Y. Tomizawa, et al. (2007). "Phase II prospective study of the efficacy of gefitinib for the treatment of stage III/IV non-small cell lung cancer with EGFR mutations, irrespective of previous chemotherapy." Lung Cancer **56**(3): 383-9.

PURPOSE: Mutations in the epidermal growth factor receptor (EGFR) gene are associated with increased sensitivity of non-small cell lung cancer (NSCLC) to gefitinib, an EGFR tyrosine kinase inhibitor. The objective of this study was to prospectively evaluate the efficacy of gefitinib in patients with stage III/IV NSCLC whose tumors carried EGFR mutations, irrespective of previous chemotherapy. EXPERIMENTAL DESIGN: Genomic DNA was extracted from tumor specimens and EGFR mutations in exons 19 and 21 analyzed by direct sequencing. Patients with stage III/IV NSCLC whose tumors had the EGFR mutations received gefitinib (250 mg/day orally). Response, toxicity and survival data were assessed. RESULT: From November 2004-May 2006, 21 patients with EGFR mutations received gefitinib (median age: 59 years; 17 females; 19 non-smokers; all had adenocarcinomas). Two patients discontinued gefitinib and withdrew from the study 3 weeks after gefitinib initiation (interstitial pneumonitis, 1 patient; facial acne, 1 patient). Of 19 patients, 3 achieved complete response, 13 exhibited partial response and 3 had stable disease. Response and disease control rates were 76% (95% confidence interval [CI] 53-92) and 90% (95% CI 70-99), respectively. The most common adverse event was skin toxicity (67%); however, no grade 4 skin toxicities were seen. Ten patients relapsed and three died at a median follow-up period of 12.6 months (range 5.6-23.8 months); median progression-free survival was 12.9 months. CONCLUSION: Analysis of tumor EGFR mutations in patients with NSCLC could be used to identify patients suitable for treatment with gefitinib to obtain optimum response and disease control rates.

Sutani, A., Y. Nagai, et al. (2006). "Gefitinib for non-small-cell lung cancer patients with epidermal growth factor receptor gene mutations screened by peptide nucleic acid-locked nucleic acid PCR clamp." Br J Cancer **95**(11): 1483-9.

This study was prospectively designed to evaluate a phase II study of gefitinib for non-small-cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutations. Clinical samples were tested for EGFR mutations by peptide nucleic acid-locked nucleic acid PCR clamp, and patients having EGFR mutations were given gefitinib 250 mg daily as the second treatment after chemotherapy. Poor PS patients omitted chemotherapy. Of 107 consecutive patients enrolled, samples from 100 patients were informative, and EGFR mutations were observed in 38 patients. Gefitinib was given to 27 patients with EGFR

mutations, and the response rate was 78% (one complete response and 20 partial responses; 95% confidence interval: 58-93%). Median time to progression and median survival time (MST) from gefitinib treatment were 9.4 and 15.4 months, respectively. Grade 3 hepatic toxicity and skin toxicity were observed in one patient each. There were significant differences between EGFR mutations and wild-type patients in response rates (78 vs 14%, P = 0.0017), and MST (15.4 vs 11.1 months, P = 0.0135). A Cox proportional hazards model indicated that negative EGFR mutation was a secondary prognostic factor (hazards ratio: 2.259, P = 0.036). This research showed the need for screening for EGFR mutations in NSCLC patients.

Suzuki, M., H. Shigematsu, et al. (2005). "Epidermal growth factor receptor expression status in lung cancer correlates with its mutation." Hum Pathol **36**(10): 1127-34.

The molecular mechanisms for frequent epidermal growth factor receptor (EGFR, a tyrosine kinase [TK]) and HER2 (the preferred coreceptor of EGFR) overexpression in lung cancer are poorly understood. Recent studies have shown the mutations of the TK domain in EGFR and HER2 to be present in lung cancer. The purpose of this study was to investigate the relationship between mutation status and expression of EGFR and HER2 in lung cancer. Immunostaining took place for EGFR and HER2, and mutational analyses for EGFR, HER2, and KRAS (a signaling protein) were conducted using 130 resected lung cancer specimens. Thirty-seven EGFR mutations (28%) and 8 HER2 mutations (6%), both of the TK domains, and 5 KRAS (4%) mutations were found, whereas 73 (56%) EGFR and 47 (36%) HER2 overexpressions were found. EGFR overexpression was seen more frequently in tumors with EGFR mutation (28/37, 76%) than in tumors without EGFR mutations (45/93, 48%; P = .0059). No correlation was found between HER2 mutation and HER2 expression. Multivariate regression revealed that EGFR mutation, adenocarcinoma histology, and HER2 expression were associated with EGFR expression, whereas female sex, EGFR mutation, and EGFR expression were associated with HER2 expression. In conclusion, EGFR and HER2 overexpression is frequent in lung cancer, and EGFR overexpression correlates with the EGFR TK domain mutations.

Tada, Y., R. M. Brena, et al. (2006). "Epigenetic modulation of tumor suppressor CCAAT/enhancer binding protein alpha activity in lung cancer." J Natl Cancer Inst **98**(6): 396-406.

BACKGROUND: Loss of tumor suppressor CCAAT/enhancer-binding protein-alpha (C/EBPalpha) expression is seen in several human malignancies, including acute myelogenous leukemia and lung cancer. We hypothesized that DNA methylation and histone acetylation of the C/EBPalpha promoter may modulate C/EBPalpha expression in lung cancer. METHODS: We analyzed C/EBPalpha expression in 15 human lung cancer cell lines and in 122 human lung primary tumors by northern blotting, immunoblotting, and immunohistochemistry. C/EBPalpha promoter methylation was assessed using bisulfite sequencing, combined bisulfite restriction analysis, methylation-specific polymerase chain reaction, and Southern blotting. We examined the acetylation status of histones H3 and H4 at the C/EBPalpha promoter by chromatin immunoprecipitation. Binding of methyl-CpG-binding proteins MeCP2 and MBD2 and upstream stimulatory factor (USF) to the C/EBPalpha promoter was assayed in cell lines that were untreated or treated with histone deacetylase inhibitor trichostatin A and demethylating agent 5-aza-2'-deoxycytidine (5-aza-dC) by chromatin immunoprecipitation and by electrophoretic mobility shift assays. RESULTS: DNA methylation and histone acetylation in the upstream region (-1422 to -896) of the C/EBPalpha promoter were associated with low or absent C/EBPalpha expression in 12 of 15 lung cancer cell lines and in 81 of 120 primary lung tumors. MeCP2 and MBD binding to the upstream C/EBPalpha promoter was detected in C/EBPalpha-nonexpressing cell lines; USF binding was detected in C/EBPalpha-expressing cell lines; however, in C/EBPalpha-nonexpressing cell lines USF binding was detected only after trichostatin A and 5-aza-dC treatment. CONCLUSIONS: DNA hypermethylation of the upstream C/EBPalpha promoter region, not the core promoter region as previously reported, is critical in the regulation of C/EBPalpha expression in human lung cancer.

Takano, T., Y. Ohe, et al. (2005). "Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer." J Clin Oncol **23**(28): 6829-37.

PURPOSE: To evaluate epidermal growth factor receptor (EGFR) mutations and copy number as predictors of clinical outcome in patients with non-small-cell lung cancer (NSCLC) receiving gefitinib. PATIENTS AND METHODS: Sixty-six patients with NSCLC who experienced relapse after surgery and received gefitinib were included. Direct sequencing of exons 18 to 24 of EGFR and exons 18 to 24 of ERBB2 was performed using DNA extracted from

surgical specimens. Pyrosequencing and quantitative real-time polymerase chain reaction were performed to analyze the allelic pattern and copy number of EGFR. RESULTS: Thirty-nine patients (59%) had EGFR mutations; 20 patients had deletional mutations in exon 19, 17 patients had missense mutations (L858R) in exon 21, and two patients had missense mutations (G719S or G719C) in exon 18. No mutations were identified in ERBB2. Response rate (82% [32 of 39 patients] v 11% [three of 27 patients]; P < .0001), time to progression (TTP; median, 12.6 v 1.7 months; P < .0001), and overall survival (median, 20.4 v 6.9 months; P = .0001) were significantly better in patients with EGFR mutations than in patients with wild-type EGFR. Increased EGFR copy numbers (> or = 3/cell) were observed in 29 patients (44%) and were significantly associated with a higher response rate (72% [21 of 29 patients] v 38% [14 of 37 patients]; P = .005) and a longer TTP (median, 9.4 v 2.6 months; P = .038). High EGFR copy numbers (> or = 6/cell) were caused by selective amplification of mutant alleles. CONCLUSION: EGFR mutations and increased copy numbers were significantly associated with better clinical outcome in gefitinib-treated NSCLC patients.

Tamura, K., I. Okamoto, et al. (2008). "Multicentre prospective phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403)." Br J Cancer **98**(5): 907-14.

The purpose of this study was to evaluate the efficacy of gefitinib and the feasibility of screening for epidermal growth factor receptor (EGFR) mutations among select patients with advanced non-small cell lung cancer (NSCLC). Stage IIIB/IV NSCLC, chemotherapy-naive patients or patients with recurrences after up to two prior chemotherapy regimens were eligible. Direct sequencing using DNA from tumour specimens was performed by a central laboratory to detect EGFR mutations. Patients harbouring EGFR mutations received gefitinib. The primary study objective was response; the secondary objectives were toxicity, overall survival (OS), progression-free survival (PFS), 1-year survival (1Y-S) and the disease control rate (DCR). Between March 2005 and January 2006, 118 patients were recruited from 15 institutions and were screened for EGFR mutations, which were detected in 32 patients--28 of whom were enrolled in the present study. The overall response rate was 75%, the DCR was 96% and the median PFS was 11.5 months. The median OS has not yet been reached, and the 1Y-S was 79%. Thus, gefitinib chemotherapy in patients with advanced NSCLC harbouring EGFR mutations was highly effective. This trial documents the feasibility of performing a multicentre phase II study using a central typing laboratory, demonstrating the benefit to patients of selecting gefitinib treatment based on their EGFR mutation status.

Tang, X., W. Wu, et al. (2004). "Hypermethylation of the death-associated protein kinase promoter attenuates the sensitivity to TRAIL-induced apoptosis in human non-small cell lung cancer cells." Mol Cancer Res **2**(12): 685-91.

Death-associated protein (DAP) kinase plays an important role in IFN-gamma, tumor necrosis factor (TNF)-alpha, or Fas-ligand induced apoptosis. TNF-related apoptosis-inducing ligand (TRAIL) is a member of the TNF ligand family and can induce caspase-dependent apoptosis in cancer cells while sparing most of the normal cells. However, some of the cancer cell lines are insensitive to TRAIL, and such resistance cannot be explained by the dysfunction of TRAIL receptors or their known downstream targets. We reported previously that DAP kinase promoter is frequently methylated in non-small cell lung cancer (NSCLC), and such methylation is associated with a poor clinical outcome. To determine whether DAP kinase promoter methylation contributes to TRAIL resistance in NSCLC cells, we measured DAP kinase promoter methylation and its gene expression status in 11 NSCLC cell lines and correlated the methylation/expression status with the sensitivity of cells to TRAIL. Of the 11 cell lines, 1 had a completely methylated DAP kinase promoter and no detectable DAP kinase expression, 4 exhibited partial promoter methylation and substantially decreased gene expression, and the other 6 cell lines showed no methylation in the promoter and normal DAP kinase expression. Therefore, the amount of DAP kinase expression amount was negatively correlated to its promoter methylation (r = -0.77; P = 0.003). Interestingly, the cell lines without the DAP kinase promoter methylation underwent substantial apoptosis even in the low doses of TRAIL, whereas those with DAP kinase promoter methylation were resistant to the treatment. The resistance to TRAIL was reciprocally correlated to DAP kinase expression in 10 of the 11 cell lines at 10 ng/mL concentration (r = 0.91; P = 0.001). We treated cells resistant to TRAIL with 5-aza-2'-deoxycytidine, a demethylating reagent, and found that these cells expressed DAP kinase and became sensitive to TRAIL. These results suggest that DAP kinase is involved in TRAIL-mediated cell apoptosis and that a demethylating agent may have a role in enhancing TRAIL-mediated apoptosis in some NSCLC cells by reactivation of DAP kinase.

Tatematsu, A., J. Shimizu, et al. (2008). "Epidermal growth factor receptor mutations in small cell lung cancer." Clin Cancer Res **14**(19): 6092-6.

PURPOSE: The vast majority of epidermal growth factor receptor (EGFR) mutations occur in lung adenocarcinoma, and even rare cases of other subtypes with this mutation, such as adenosquamous cell carcinoma, are associated with adenocarcinoma histology. According to this adenocarcinoma-specific nature of EGFR mutation, analysis of EGFR mutations with small cell lung cancers (SCLC) may provide a clue to its histogenesis. EXPERIMENTAL DESIGN: The mutational status of the EGFR gene was accessed in a cohort of 122 patients with SCLC; all patients were from a single institute. When the EGFR mutated, its gene copy number was also examined. RESULTS: EGFR mutations were detected in five SCLCs (4%). The patients were mainly in the light smoker and histologic combined subtype. All but one of the tumors harbored gene amplifications. Notably, in three tumors of the combined SCLC subtype, both components of adenocarcinoma and SCLC harbored an EGFR mutation, whereas gene amplification was detected only in the adenocarcinoma component. A partial response was achieved in a patient (with an EGFR mutation) who was treated with gefitinib. CONCLUSIONS: Although EGFR mutations are rare in SCLC, a combined subtype of SCLC with adenocarcinoma in light smokers may have a chance of harboring EGFR mutations. For patients with an EGFR mutation, EGFR tyrosine kinase inhibitor can be a treatment option. In terms of molecular pathogenesis, it is suggested that some SCLCs may have developed from pre-existing adenocarcinomas with EGFR mutations, but the development may not be simply linear, taking into consideration the discordant distribution of EGFR amplification.

Tiseo, M., M. Capelletti, et al. (2008). "Epidermal growth factor receptor intron-1 polymorphism predicts gefitinib outcome in advanced non-small cell lung cancer." J Thorac Oncol **3**(10): 1104-11.

INTRODUCTION: Epidermal growth factor receptor (EGFR) gene intron 1 contains a polymorphic single sequence dinucleotide repeat (CA)n whose length has been found to inversely correlate with transcriptional activity. This study was designed to assess the role of (CA)n polymorphism in predicting the outcome of gefitinib treatment in advanced non-small cell lung cancer (NSCLC). METHODS: Blood and tumor tissue from 58 patients with advanced NSCLC submitted to gefitinib were collected. EGFR intron 1 gene polymorphism, along with EGFR gene mutation, gene copy number and immunohistochemistry expression were determined. Moreover, a panel of lung cancer cell lines characterized for EGFR intron 1 polymorphism was also studied. RESULTS: EGFR intron 1 polymorphism showed a statistically significant correlation with the gefitinib response (response rate 25 versus 0%, for patients with a (CA)16 and with a (CA)else genotype, respectively; p = 0.044). Patients with a (CA)16 genotype had a longer survival compared with those with a (CA)else genotype (11.4 versus 4.8 months, respectively; p = 0.037). In addition, cell lines lacking the (CA)16 allele showed a statistically significant higher IC50 compared with cell lines bearing at least one (CA)16 allele (p = 0.003). CONCLUSIONS: This study supports a potential role of EGFR intron 1 polymorphism in predicting the outcome of gefitinib treatment in advanced NSCLC.

Tokumo, M., S. Toyooka, et al. (2006). "Double mutation and gene copy number of EGFR in gefitinib refractory non-small-cell lung cancer." Lung Cancer **53**(1): 117-21.

Mutations of the epidermal growth factor receptor (EGFR) gene have been reported in non-small-cell lung cancer (NSCLC), especially in patients with adenocarcinoma and never smokers. Some common somatic mutations in EGFR, including deletion mutations in exon 19 and leucine-to-arginine substitution at amino acid position 858 (L858R) in exon 21, have been examined for their ability to predict sensitivity to gefitinib or erlotinib, which are selective EGFR tyrosine kinase inhibitors (EGFR-TKIs). On the other hand, reports have shown that the threonine-to-methionine substitution at amino acid position 790 (T790M) in exon 20 is related to gefitinib resistance. Some studies have indicated that high copy numbers of the EGFR gene may be a more effective molecular predictor to responsiveness and prolonged survival in patients treated with EGFR-TKIs. Here, we describe two NSCLC patients with the L858R mutation who did not respond to gefitinib. Case 1 harbored both the T790M and L858R mutations, and fluorescence in situ hybridization showed EGFR gene amplification. Case 2 harbored both the L858R and aspartic acid-to-tyrosine substitution at amino acid position 761 in exon 19 of EGFR mutations and had a high polysomy status for EGFR. In these two cases, tumors showed resistance to gefitinib treatment despite the presence of EGFR L858R mutation and increased copy number. Our findings encourage further molecular analysis to elucidate the relationship between the EGFR status, including mutations and amplifications, and the responsiveness of NSCLC to gefitinib.

Tomizawa, Y., H. Iijima, et al. (2005). "Clinicopathologic significance of the mutations of the epidermal growth factor receptor gene in patients with non-small cell lung cancer." Clin Cancer Res **11**(19 Pt 1): 6816-22.

PURPOSE: It has been reported that the mutations of epidermal growth factor receptor (EGFR) are detected in lung cancers. Studies of EGFR mutations in large numbers of patients' tumors with clinical data including response to EGFR tyrosine kinase directed therapy are needed to develop a robust database for clinical use. The purpose of the present study is to gain further insights into the significance of EGFR mutation in non-small cell lung cancer (NSCLC). EXPERIMENTAL DESIGN: We investigated the clinicopathologic significance of tyrosine kinase domain (exons 18-21) EGFR mutations in 120 patients with primary NSCLC and the correlation between EGFR mutation and sensitivity to gefitinib in an additional 20 NSCLC patients treated with gefitinib. In addition, onocogenic KRAS mutations and RASSF1A promoter methylation were determined in the same samples. RESULTS: EGFR mutation was detected in 29 of 120 (24%) tumors. All of the 29 (40%) mutations occurred in 72 adenocarcinomas. EGFR mutation was significantly more frequent in females (47%) than males (12%, $P < 0.0001$), in younger patients (38%) than older patients (10%, $P = 0.0005$), in nonsmokers (47%) than smokers (13%, $P < 0.0001$), and in well-differentiated tumors (39%) than moderately and poorly differentiated tumors (7%, $P < 0.0001$). Mutation of the EGFR gene was preferentially observed in advanced disease. Furthermore, EGFR mutations were detected in 11 of 14 (79%) responders, whereas none of six (0%) nonresponders had the mutation ($P = 0.0022$). CONCLUSIONS: These results in Japanese (East Asian) patients indicated that EGFR mutation plays an important role in pathogenesis of lung adenocarcinoma.

Toyooka, S., A. Uchida, et al. (2007). "The effect of gefitinib on B-RAF mutant non-small cell lung cancer and transfectants." J Thorac Oncol **2**(4): 321-4.

We previously reported one patient with squamous cell carcinoma of the lung that showed the long-term effect to gefitinib with complete response. In the present report, we examine the epidermal growth factor receptor (EGFR) and K-RAS, HER2, and B-RAF mutations in this patient to find a B-RAF exon11 mutation, resulting in a substitution of valine by phenylalanine at codon 470 (V470F) as a novel type of B-RAF mutation in human cancers. In addition, the fluorescence in situ hybridization analysis for EGFR showed the high polysomy status. B-RAF is a nonreceptor serine/threonine kinase whose kinase domain has a structure similar to other protein kinases, including EGFR members. Of interest, the B-RAF V470F mutation corresponds to a position similar to the EGFR G719X mutation located on the phosphate binding (P)-loop of EGFR that clamps ATP into the catalytic cleft. This observation suggests that gefitinib may have an anti-cancer effect on B-RAF mutant tumors. Indeed, previous reports demonstrated that H1666 cells harboring B-RAF G465V mutations showed sensitivity to gefitinib, inhibiting phosphorylation of ERK1/2. We examined the effect of gefitinib on transient transfectants of the B-RAF mutant, but no drastic inhibition of ERK1/2 phosphorylation that was one of the downstream molecules of B-RAF was induced by gefitinib. In summary, we found a novel B-RAF V470F mutation in lung squamous cell carcinoma that showed response to gefitinib. However, our in vitro investigation did not explain the response observed in this particular patient. Further investigation is necessary to elucidate the mechanism of tumor sensitivity to EGFR tyrosine kinase inhibitors.

Tsao, M. S., A. Sakurada, et al. (2005). "Erlotinib in lung cancer - molecular and clinical predictors of outcome." N Engl J Med **353**(2): 133-44.

BACKGROUND: A clinical trial that compared erlotinib with a placebo for non-small-cell lung cancer demonstrated a survival benefit for erlotinib. We used tumor-biopsy samples from participants in this trial to investigate whether responsiveness to erlotinib and its impact on survival were associated with expression by the tumor of epidermal growth factor receptor (EGFR) and EGFR gene amplification and mutations. METHODS: EGFR expression was evaluated immunohistochemically in non-small-cell lung cancer specimens from 325 of 731 patients in the trial; 197 samples were analyzed for EGFR mutations; and 221 samples were analyzed for the number of EGFR genes. RESULTS: In univariate analyses, survival was longer in the erlotinib group than in the placebo group when EGFR was expressed (hazard ratio for death, 0.68; $P=0.02$) or there was a high number of copies of EGFR (hazard ratio, 0.44; $P=0.008$). In multivariate analyses, adenocarcinoma ($P=0.01$), never having smoked ($P<0.001$), and expression of EGFR ($P=0.03$) were associated with an objective response. In multivariate analysis, survival after treatment with erlotinib was not influenced by the status of EGFR expression, the number of EGFR copies, or EGFR mutation. CONCLUSIONS: Among patients with non-small-cell lung cancer who receive erlotinib, the presence of an EGFR mutation may increase responsiveness to the agent, but it is not indicative of a survival benefit.

Uchida, K., A. Kojima, et al. (2002). "Expression of progastrin-releasing peptide and gastrin-releasing peptide receptor mRNA transcripts in tumor cells of patients with small cell lung cancer." J Cancer Res Clin Oncol **128**(12): 633-40.

PURPOSE: Small cell lung cancer (SCLC) is a rapidly growing neoplasm accounting for approximately 20% of patients with lung cancer. Progastrin-releasing peptide (proGRP) is produced in about two-thirds of SCLC tumors and is used as a specific marker for SCLC. Although GRP is known to have a variety of biological functions, only limited information is available concerning expression of proGRP mRNA and protein, and that of the receptor for GRP (GRPR) in SCLC tumors. METHODS: In individuals with SCLC, the levels of serum proGRP(31-98) were measured by enzyme-linked immunosorbent assay. Expression of proGRP as well as GRPR mRNA in SCLC tumor tissues was investigated by reverse transcription-nested polymerase chain reaction (PCR) amplification. The proportions of alternatively spliced proGRP mRNA transcripts were analyzed in proGRP-producing tumors by nested and competitive PCR amplification. Finally, production of proGRP protein in SCLC tumor was evaluated by using immunohistochemical staining with a polyclonal human anti-proGRP antibody. RESULTS: ProGRP mRNA transcripts could be detected only in tumor tissues recovered from individuals with high serum proGRP levels. The proportions of mRNA subtypes in each case were nearly the same, revealing type I of 55.4+/-7.6%, type II with 21-b deletion of 1.8+/-3.6%, and type III with 19-b deletion of 42.8+/-4.3%, respectively. ProGRP protein production was demonstrated in tumor tissues exclusively from individuals exhibiting high serum proGRP levels. In contrast, GRPR mRNA transcripts were detectable in cancer cells from two of five proGRP-expressing tumor tissues. CONCLUSIONS: ProGRP mRNA expression is closely related with the synthesis of proGRP protein which is eventually released into the blood. It is suggested GRP may function as an autocrine growth factor for cancer cells in a subgroup of SCLC patients through, at least in part, upregulation of GRPR expression.

Ugocsai, K., L. Mandoky, et al. (2005). "Investigation of HER2 overexpression in non-small cell lung cancer." Anticancer Res **25**(4): 3061-6.

Lung cancer is the leading cause of mortality worldwide. The median survival of advanced disease is in the range of 8 to 10 months. Intrinsic or acquired drug resistance pose major challenges to the success of chemotherapy. The HER2 gene, also known as c-erbB-2 or neu, is a proto-oncogene that encodes a membrane-bound receptor tyrosine kinase of the epithelial growth factor receptor (EGFR) family. It has a possible role in tumor cell proliferation, tumor invasion, tumor metastasis and drug resistance. We retrospectively investigated 88 samples of non-small cell lung cancer (NSCLC) and assessed the correlation between HER2 expression and tumor histology. The expression of HER2 protein was analyzed by immunohistochemical staining (IHC) and HER2 DNA amplification was detected by using fluorescence in situ hybridization (FISH). HER2 overexpression (2+, 3+) was detected in 5 (5.7%) out of 88 specimens. All of the HER2-overexpressing tumors histologically proved to be squamous cell carcinoma (SCC). Cases with 2+ HER2 immunoreactivity showed either no amplification (3.875 and 2.525), or borderline amplification (4.75). Cases with 3+ HER2 immunoreactivity showed moderate amplification (7.35) and strong amplification (15-20 - cluster), respectively. The HER2 expression in NSCLC was relatively low in the selected Hungarian population; consequently, there is no indication for introduction of trastuzumab for the treatment of lung cancer.

Uramoto, H., K. Sugio, et al. (2004). "Expression of deltaNp73 predicts poor prognosis in lung cancer." Clin Cancer Res **10**(20): 6905-11.

PURPOSE: DeltaNp73 is an isoform of the p53 homologue p73, which lacks an NH(2)-terminal transactivation domain and antagonizes the induction of gene expression by p53/p73. The aim of this study was to detect DeltaNp73 expression in lung cancer and to evaluate the relationship between the DeltaNp73 expression level and the prognosis of patients with resected lung cancer. EXPERIMENTAL DESIGN: We used immunohistochemistry to analyze the protein expression of DeltaNp73 in paraffin-embedded tumor samples from 132 well-characterized lung cancer patients and compared the expression level of DeltaNp73, clinical variables, and survival outcome. RESULTS: Positive expression of DeltaNp73 was detected mainly in the cytoplasm of tumor cells in 77 of 132 patients (58.3%) with lung cancer. The incidence of positive expression of DeltaNp73 was 52.2, 50.0, and 70.2% in patients with stage I, II, and III, respectively ($P = 0.04$). Positive expression of DeltaNp73 was associated with gender but not associated with age, histologic type, pathological stage, pathological T status, and pathological N status. Lung cancer patients with positive DeltaNp73 expression had a poorer prognosis than those with negative DeltaNp73 expression. In addition, multivariate analysis of the clinicopathological characteristics of lung cancer indicated that positive expression of DeltaNp73 was a significant independent factor for predicting poor prognosis ($P < 0.0001$, risk ratio = 3.39).

CONCLUSIONS: Expression of DeltaNp73 may be a useful marker for predicting poor prognosis of patients who underwent resection of lung cancer.

Uramoto, H., K. Sugio, et al. (2006). "Expression of the p53 family in lung cancer." Anticancer Res **26**(3A): 1785-90.

BACKGROUND: p53 is mutated in about 50% of various malignant diseases including lung cancer. The p53 family consists of p53, p73 and p63. Although transactivating protein isoforms display p53-like functions, the deltaNp73 or deltaNp63 isoforms act toward p53 in a dominantly negative way. The aim of this study was to detect p53, deltaNp73 and deltaNp63 expressions in lung cancer and to evaluate the relationship between the expression levels of the proteins and the prognosis of patients with resectable lung cancer. MATERIALS AND METHODS: Immunohistochemistry was employed to analyze the protein expression of p53, deltaNp73 and deltaNp63 in paraffin-embedded tumor samples from 132 well-characterized lung cancer patients. The correlation among the expression levels of p53, deltaNp73 and deltaNp63, clinical variables and survival outcome was analyzed. RESULTS: Positive expressions of p53, deltaNp73 and deltaNp63 were detected in the tumor cells in 52, 77 and 44 of the 132 patients, respectively (39.4%, 58.3% and 33.3%) with lung cancer. The incidence of p53 positive expression was 54.5% and 27.6% in patients with squamous cell carcinoma and adenocarcinoma, respectively ($p = 0.03$). The incidence of a positive expression of deltaNp73 was 64.5% and 43.6% in male and female patients, respectively ($p = 0.03$). The incidence of deltaNp63 positive expression was 68.2% and 15.8% in the patients with squamous cell carcinoma and adenocarcinoma, respectively ($p < 0.0001$). The expressions of p53 and deltaNp63 were not found to significantly affect survival. However, lung cancer patients with a positive deltaNp73 expression had a poorer prognosis than those with a negative deltaNp73 expression. In addition, multivariate analysis indicated that a positive expression of deltaNp73 was a significantly independent factor for predicting a poor prognosis ($p < 0.0001$, risk ratio = 3.38). CONCLUSION: Clinical evidence that the p53 family is frequently overexpressed in lung cancer specimens, especially deltaNp63 in squamous cell carcinoma, was provided. The expression of deltaNp73 may be a useful marker for predicting a poor prognosis in resectable lung cancer. Understanding how groups of lung cancer cell genes are coordinately expressed in response to physiological, immunological and micro-environmental stimuli remains an important goal. A better understanding of the gene expression profiles of tumors may help to identify molecular targets, such as deltaNp73, for effective therapy.

Uramoto, H., K. Sugio, et al. (2006). "Epidermal growth factor receptor mutations are associated with gefitinib sensitivity in non-small cell lung cancer in Japanese." Lung Cancer **51**(1): 71-7.

The protein-kinase family is the most frequently mutated gene family found in human cancer. Gefitinib, an ATP-competitive inhibitor of epidermal growth factor receptor (EGFR), also appears to be particularly effective in adenocarcinoma of the lung and in patients without smoking history. To determine whether lung tumors sensitive to gefitinib contained mutations within the tyrosine kinase (TK) domain of EGFR, we screened exons 18-23 of EGFR of tumors in 20 patients with non-small cell lung cancer (NSCLC) who had been treated with gefitinib. Nine (45%) tumors had TK domain mutations. All mutations were observed in adenocarcinoma. Seven (77.8%) of 9 cases with mutated types showed sensitivity to gefitinib, while no cases of 11 with wild type showed gefitinib sensitivity. Such mutations were more frequently observed in patients who had never smoked (5/8 or 62.5%) than in smokers (4/12 or 33.3%). The patients with mutations of EGFR to have a more favorable prognosis than those with wild type ($p=0.033$). These data show that adenocarcinomas from patients who had never smoked comprise a specific subset of patients with NSCLC sensitive to gefitinib treatment.

van Zandwijk, N., A. Mathy, et al. (2007). "EGFR and KRAS mutations as criteria for treatment with tyrosine kinase inhibitors: retro- and prospective observations in non-small-cell lung cancer." Ann Oncol **18**(1): 99-103.

Results of individualized therapy guided by mutational tumor profile of patients with non-small-cell lung cancer are presented. After confirming the importance of epidermal growth factor receptor (EGFR) and KRAS mutations for (non)response on gefitinib in a retrospective series of patients, EGFR mutations were looked for before--and were a condition for--treatment with gefitinib or erlotinib. To increase the chance to find such a mutation, we selected patients on the basis of smoking status, gender and histopathology. Out of 41 patients selected, 13 (32%) were found to harbor an EGFR mutation. In nine of them it concerned deletions in exon 19 and in none of them KRAS mutations were detected. All nine patients with an exon 19 deletion had a favorable and continuing response to tyrosine kinase inhibitors (TKIs), while four other patients with point mutations responded less favorably: stable disease or a response of short duration. These

observations confirm the potential role of EGFR and KRAS mutations in predicting (non)response to TKIs. Exon 19 deletions that are associated with the best responses might be used for first-line treatment selection, while KRAS mutations could play a role in excluding patients from treatment with TKIs.

Varella-Garcia, M. (2006). "Stratification of non-small cell lung cancer patients for therapy with epidermal growth factor receptor inhibitors: the EGFR fluorescence in situ hybridization assay." Diagn Pathol **1**: 19.

DNA fluorescence in situ hybridization (FISH) technology is used to study chromosomal and genomic changes in fixed cell suspensions and tissue block preparations. The technique is based on specific hybridization of small labeled DNA fragments, the probes, to complementary sequences in a target DNA molecule. Demand for FISH assays in formalin-fixed, paraffin-embedded tissues has been increasing, mainly in conditions in which diagnosis is not achieved in cell smears or tissue imprints, such as solid tumors. Moreover, the development of molecular targeted therapies in oncology has expanded the applicability of tests to predict sensitivity or resistance to these agents. The efficient use of tyrosine kinase inhibitors (TKI) of the epidermal growth factor receptor (EGFR) as therapeutical agents in advanced non-small cell lung cancer (NSCLC) depends on identification of patients likely to show clinical benefit from these specific treatments. The EGFR gene copy number determined by FISH has been demonstrated as an effective predictor of outcome from NSCLC patients to EGFR TKIs; however there are pending challenges for standardization of laboratory procedures and definition of the scoring system. This methodology article focuses on the EGFR FISH assay. It details the scoring system used in the studies conducted at the University of Colorado Cancer Center in which a significant association was found between increased EGFR copy numbers and clinical outcome to TKIs, and proposes interpretative guidelines for molecular stratification of NSCLC patients for TKI therapy.

Varella-Garcia, M., T. Mitsudomi, et al. (2009). "EGFR and HER2 genomic gain in recurrent non-small cell lung cancer after surgery: impact on outcome to treatment with gefitinib and association with EGFR and KRAS mutations in a Japanese cohort." J Thorac Oncol **4**(3): 318-25.

BACKGROUND: Sensitivity to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) and frequency of activation mutations in EGFR is lower in Caucasian than Asian non small-cell lung cancer (NSCLC) patients. Increased EGFR gene copy numbers evaluated by fluorescence in situ hybridization (FISH) has been reported as predictor of clinical benefit from EGFR-TKIs in Caucasian NSCLC patients. This study was carried out to verify whether EGFR FISH had similar performance in Japanese patients. METHODS: A cohort of 44 Japanese patients with recurrent NSCLC after surgery was treated with gefitinib 250 mg daily. The cohort included 48% females and 52% never-smokers; 73% had prior chemotherapy and 57% had stage III-IV at the time of surgery. Adenocarcinoma was the most common histology (86%). FISH was performed using the EGFR/Chromosome Enumeration Probe 7 and PathVysion DNA probes (Abbott Molecular). Specimens were classified as FISH positive when showing gene amplification or high polysomy (> or = 4 copies of the gene in > or = 40% of tumor cells). Tumor response to gefitinib was assessed by RECIST for 33 patients with measurable diseases. RESULTS: Twenty-nine tumors (66%) were EGFR FISH+ and 23 (53%) were HER2 FISH+. Overall response rate was 52%, representing 65% of EGFR FISH+ patients and 29% of EGFR FISH- patients ($p = 0.0777$). Survival was not impacted by the EGFR FISH ($p = 0.9395$) or the HER2 FISH ($p = 0.0671$) status. EGFR FISH+ was significantly associated with HER2 FISH+ ($p = 0.015$) and presence of EGFR mutation ($p = 0.0060$). EGFR mutation significantly correlated with response ($p < 0.0001$) and survival after gefitinib ($p = 0.0204$). EGFR and HER2 FISH status were not associated with KRAS mutation. CONCLUSION: Frequency of EGFR FISH+ status was higher and its predictive power for TKI sensitivity was lower in this Japanese cohort than in Western NSCLC cohorts. These findings support differences in the mechanisms of EGFR pathway activation in NSCLC between Asian and Caucasian populations. Confirmation of these results in larger cohorts is warranted.

Vidaver, R. M. and B. S. Schachter (2009). "2008 Meeting of the National Lung Cancer Partnership: a summary of meeting highlights." J Thorac Oncol **4**(5): 666-8.

Herein are highlights from National Lung Cancer Partnership's Annual Meeting, held May 30, 2008 in Chicago. Aiming to improve the match between lung cancer patients and their drug treatments, speakers described potential predictive and prognostic biomarkers. Approaches included: (1) in non-small cell lung cancer, testing for predictive links between tumor expression levels of DNA synthesis (RRM1) and repair (ERCC1) enzymes and response to gemcitabine and cisplatin respectively, and looking for a prognostic link with ERCC1 expression; (2) validating a predictive "meta-gene profile" from gene expression microarray studies to distinguish drug-

responsive from unresponsive lung cancer tumors; and (3) developing proteomics profiling to distinguish lung cancer patients, including squamous and nonsquamous cell carcinoma patients, who respond to epidermal growth factor receptor tyrosine kinase inhibitors from those who do not. The notion that cancer stem cells are fundamental in the development and progression of solid tumors including lung cancers was also discussed. Potential strategies for using this information to identify useful targets for next-generation therapies were suggested.

Vikis, H., M. Sato, et al. (2007). "EGFR-T790M is a rare lung cancer susceptibility allele with enhanced kinase activity." Cancer Res **67**(10): 4665-70.

The use of tyrosine kinase inhibitors (TKI) has yielded great success in treatment of lung adenocarcinomas. However, patients who develop resistance to TKI treatment often acquire a somatic resistance mutation (T790M) located in the catalytic cleft of the epidermal growth factor receptor (EGFR) enzyme. Recently, a report describing EGFR-T790M as a germ-line mutation suggested that this mutation may be associated with inherited susceptibility to lung cancer. Contrary to previous reports, our analysis indicates that the T790M mutation confers increased Y992 and Y1068 phosphorylation levels. In a human bronchial epithelial cell line, overexpression of EGFR-T790M displayed a growth advantage over wild-type (WT) EGFR. We also screened 237 lung cancer family probands, in addition to 45 bronchoalveolar tumors, and found that none of them contained the EGFR-T790M mutation. Our observations show that EGFR-T790M provides a proliferative advantage with respect to WT EGFR and suggest that the enhanced kinase activity of this mutant is the basis for rare cases of inherited susceptibility to lung cancer.

Volm, M., R. Koomagi, et al. (2002). "Protein expression profiles indicative for drug resistance of non-small cell lung cancer." Br J Cancer **87**(3): 251-7.

Data obtained from multiple sources indicate that no single mechanism can explain the resistance to chemotherapy exhibited by non-small cell lung carcinomas. The multi-factorial nature of drug resistance implies that the analysis of comprising expression profiles may predict drug resistance with higher accuracy than single gene or protein expression studies. Forty cellular parameters (drug resistance proteins, proliferative, apoptotic, and angiogenic factors, products of proto-oncogenes, and suppressor genes) were evaluated mainly by immunohistochemistry in specimens of primary non-small cell lung carcinoma of 94 patients and compared with the response of the tumours to doxorubicin in vitro. The protein expression profile of non-small cell lung carcinoma was determined by hierarchical cluster analysis and clustered image mapping. The cluster analysis revealed three different resistance profiles. The frequency of each profile was different (77, 14 and 9%, respectively). In the most frequent drug resistance profile, the resistance proteins P-glycoprotein/MDR1 (MDR1, ABCB1), thymidylate-synthetase, glutathione-S-transferase-pi, metallothionein, O6-methylguanine-DNA-methyltransferase and major vault protein/lung resistance-related protein were up-regulated. Microvessel density, the angiogenic factor vascular endothelial growth factor and its receptor FLT1, and ECGF1 as well were down-regulated. In addition, the proliferative factors proliferating cell nuclear antigen and cyclin A were reduced compared to the sensitive non-small cell lung carcinoma. In this resistance profile, FOS was up-regulated and NM23 down-regulated. In the second profile, only three resistance proteins were increased (glutathione-S-transferase-pi, O6-methylguanine-DNA-methyltransferase, major vault protein/lung resistance-related protein). The angiogenic factors were reduced. In the third profile, only five of the resistance factors were increased (MDR1, thymidylate-synthetase, glutathione-S-transferase-pi, O6-methylguanine-DNA-methyltransferase, major vault protein/lung resistance-related protein).

Wang, L., J. C. Soria, et al. (2002). "hTERT expression is a prognostic factor of survival in patients with stage I non-small cell lung cancer." Clin Cancer Res **8**(9): 2883-9.

Activation of telomerase plays a critical role in unlimited proliferation and immortalization of cells. The purpose of this study was to evaluate the significance of human telomerase reverse transcriptase catalytic subunit (hTERT) as a prognostic marker. The expression of hTERT in a large population of 153 patients with stage I non-small cell lung cancer was analyzed using the in situ hybridization technique. We found that diffuse and clear hTERT expression was present in 51 (33%) of 153 patients. Kaplan-Meier analysis showed that hTERT expression was associated with shorter overall survival ($P = 0.04$), shorter disease-specific survival ($P = 0.03$), and shorter disease-free survival ($P = 0.02$). Multivariate analysis confirmed this independent prognostic value of hTERT expression. Our results indicated that hTERT mRNA expression is associated with malignant tumor progression and poor outcome. hTERT may serve as a useful marker to identify patients with poor prognosis and to select patients with early-stage non-small cell lung cancer who might benefit from adjuvant treatment.

Watanabe, T., M. Hioki, et al. (2006). "Histone deacetylase inhibitor FR901228 enhances the antitumor effect of telomerase-specific replication-selective adenoviral agent OBP-301 in human lung cancer cells." Exp Cell Res **312**(3): 256-65.

Replication-competent oncolytic viruses are being developed for human cancer therapy. We previously reported that an attenuated adenovirus OBP-301 (Telomelysin), in which the human telomerase reverse transcriptase promoter element drives expression of E1A and E1B genes linked with an internal ribosome entry site, could replicate in and causes selective lysis of human cancer cells. Infection efficiency in target cancer cells is the most important factor that predicts the antitumor effects of OBP-301. The objectives of this study are to examine the effects of the histone deacetylase inhibitor FR901228 on the level of coxsackie and adenovirus receptor (CAR) expression and OBP-301-mediated oncolysis in human non-small cell lung cancer cell lines. Flow cytometric analysis revealed up-regulated CAR expression in A549 and H460 cells following treatment with 1 ng/ml of FR901228, which was associated with increased infection efficiency as confirmed by replication-deficient beta-galactosidase-expressing adenovirus vector. In contrast, neither CAR expression nor infection efficiency was affected by FR901228 in H1299 cells. To visualize and quantify viral replication in the presence of FR901228, we used OBP-401 (Telomelysin-GFP) that expresses the green fluorescent protein (GFP) reporter gene under the control of the cytomegalovirus promoter in the E3 region. Fluorescence microscopy and flow cytometry showed that FR901228 increased GFP expression in A549 and H460 cells following OBP-401 infection in a dose-dependent manner, but this effect did not occur in H1299 cells. In addition, OBP-301 and FR901228 demonstrated a synergistic antitumor effect in A549 cells in vitro, as confirmed by isobologram analysis. Our data indicate that FR901228 preferentially increases adenovirus infectivity via up-regulation of CAR expression, leading to a profound oncolytic effect, which may have a significant impact on the outcome of adenovirus-based oncolytic virotherapy.

Wei, H., R. Sun, et al. (2003). "Traditional Chinese medicine Astragalus reverses predominance of Th2 cytokines and their up-stream transcript factors in lung cancer patients." Oncol Rep **10**(5): 1507-12.

Th2 cytokine is predominant in tumor patients and was found to be associated with tumor progression. Reversing of Th2 dominant status is thought to be a promising strategy. In the present study, peripheral blood mononuclear cells (PBMNC) of 37 lung cancer patients and 19 healthy subjects were prepared and used for examination of cytokine secretion and gene expression. The positive percentage of mRNA transcripts of Th1 cytokines (8.1% for IFNgamma and 13.5% for IL-2) in patients' PBMNC were lower than those of Th2 cytokines (70.3% for IL-4, 64.9% for IL-6 and 83.8% for IL-10). The gene expression capacity (measured as relative intensity to ratio of beta-actin) of patients for Th1 cytokines was low, but constitutively relatively high for Th2 cytokines. Both positive percentage and relative intensity were lower in transcript factor for Th1 cytokine, T-bet (40.5% and 0.139, respectively) than those for Th2 cytokine, GATA3 (89.2% and 0.364, respectively). Traditional Chinese medicine, Astragalus (AG) was observed to reverse Th2 status of lung cancer. AG enhanced culture supernatant and gene expression levels of Th1 cytokine (IFNgamma and IL-2) and its transcript factor (T-bet), and reduced those of Th2 cytokines in cultured PBMNC of lung cancer patients. These results demonstrated that traditional Chinese medicine AG might reverse the Th2 predominant status in lung cancer patients, which is a probable alternative therapeutic regime in future.

Willmore-Payne, C., J. A. Holden, et al. (2008). "The use of EGFR exon 19 and 21 unlabeled DNA probes to screen for activating mutations in non-small cell lung cancer." J Biomol Tech **19**(3): 217-24.

Activating mutations in epidermal growth factor receptor-1 (EGFR) are found in 10-15% of Caucasian patients with non-small cell lung carcinoma (NSCLC). Approximately 90% of the mutations are deletions of several amino acids in exon 19 or point mutations in exon 21. Some studies suggest that these mutations identify patients that might benefit from targeted EGFR inhibitor therapy. DNA melting analysis of polymerase chain reaction products can screen for these mutations to identify this patient population. However, amplicon DNA melting analysis, although easily capable of detecting heterozygous mutations by heterodimer formation, becomes more difficult if mutations are homozygous or if the mutant allele is selectively amplified over wild type. Amplification of EGFR is common in NSCLC and this could compromise mutation detection by amplicon melting analysis. To overcome this potential limitation, we developed unlabeled, single-stranded DNA probes, complimentary to EGFR exon 19 and exon 21 where the common activating mutations occur. The unlabeled probes are incorporated into a standard polymerase chain reaction during the amplification of EGFR exons 19 and 21. The probe melting peak is easily distinguished from the amplicon melting peak, and probe melting is altered if mutations are present. This allows for easy

identification of activating mutations even in homozygous or amplified states and is useful in the screening of NSCLC for the common EGFR activating mutations.

Witta, S. E., R. Dziadziuszko, et al. (2009). "ErbB-3 expression is associated with E-cadherin and their coexpression restores response to gefitinib in non-small-cell lung cancer (NSCLC)." Ann Oncol **20**(4): 689-95.

BACKGROUND: Epidermal growth factor receptor (EGFR) inhibitors are effective in a subset of patients with non-small-cell lung cancer (NSCLC). We previously showed that E-cadherin expression associates with gefitinib activity. Here, we correlated the expressions of ErbB-3 and E-cadherin in NSCLC tumors and cell lines, their effect on response to gefitinib, and induction of both by the histone deacetylase (HDAC) inhibitors vorinostat and SNDX-275. METHODS: Real-time RT-PCR was carried out on RNA isolated from 91 fresh-frozen NSCLC samples and from 21 NSCLC lines. Protein expression was evaluated with western blot and flow cytometry. Apoptosis was assessed using vibrant apoptosis assay. RESULTS: Expressions of E-cadherin and ErbB-3 correlated significantly in primary tumors ($r = 0.38$, $P < 0.001$) and in cell lines ($r = 0.88$, $P < 0.001$). Cotransfection of ErbB-3 and E-cadherin in a gefitinib-resistant cell line showed enhanced apoptotic response to gefitinib. vorinostat and SNDX-275 induced ErbB-3 and E-cadherin in gefitinib-resistant cell lines. When gefitinib-resistant lines were treated with vorinostat and gefitinib, synergistic effects were detected in four of the five lines tested. CONCLUSION: ErbB-3 and E-cadherin are coexpressed and induced by HDAC inhibitors. For tumors with low ErbB-3 and E-cadherin expressions, the combination of HDAC and EGFR-tyrosine kinase inhibitors increased expression of both genes and produced more than additive apoptotic effect.

Yamamoto, H., S. Toyooka, et al. (2009). "Impact of EGFR mutation analysis in non-small cell lung cancer." Lung Cancer **63**(3): 315-21.

The discovery of mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) accelerated the research of molecular-targeted therapy by EGFR-tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib. About 90% of EGFR mutations are clustered in exons 19 (deletion) and 21 (point mutation at codon 858) and patients with these mutations have great response to EGFR-TKIs. However, tumors that initially respond to EGFR-TKIs almost inevitably become resistant later and T790M secondary mutation in the EGFR gene and MET amplification are reported to account for the mechanism of this acquired resistance. In this review, we summarize the recent findings about EGFR mutations, amplification, alterations of other related genes and sensitivity and acquired resistance to EGFR-TKIs. We also discuss from our studies the relationship between EGFR mutations and other molecular alterations such as aberrant methylation in tumor suppressor genes (TSGs), which indicates that they are related to the mechanism of the pathogenesis of lung cancer. The accumulated important data confer further insights on translational research, providing us with the new strategies for the treatment of NSCLCs.

Yan, Y., Y. Lu, et al. (2006). "Effect of an epidermal growth factor receptor inhibitor in mouse models of lung cancer." Mol Cancer Res **4**(12): 971-81.

Gefitinib (Iressa, ZD1839) is a potent high-affinity competitive tyrosine kinase inhibitor aimed primarily at epidermal growth factor receptor (EGFR). Inhibitors in this class have recently been approved for clinical use in the treatment of advanced non-small cell lung cancer as monotherapy following failure of chemotherapy. We examined the efficacy of gefitinib on lung tumorigenesis in mouse models using both postinitiation and progression protocols. Gefitinib was given at a dose of 200 mg/kg body weight (i.g.) beginning either 2 or 12 weeks following carcinogen initiation. In the postinitiation protocol, gefitinib significantly inhibited both tumor multiplicity (approximately 70%) and tumor load (approximately 90%) in A/J or p53-mutant mice ($P < 0.0001$). Interestingly, gefitinib was also highly effective against lung carcinogenesis in the progression protocol when individual animals already have multiple preinvasive lesions in the lung. Gefitinib exhibited approximately 60% inhibition of tumor multiplicity and approximately 80% inhibition of tumor load when compared with control mice (both $P < 0.0001$). These data show that gefitinib is a potent chemopreventive agent in both wild-type and p53-mutant mice and that a delayed administration was still highly effective. Analyses of mutations in the EGFR and K-ras genes in lung tumors from either control or treatment groups showed no mutations in EGFR and consistent mutation in K-ras. Using an oligonucleotide array on control and gefitinib-treated lesions showed that gefitinib treatment failed to alter the activity or the expression level of EGFR. In contrast, gefitinib treatment significantly altered the expression of a series of genes involved in cell cycle, cell proliferation, cell transformation, angiogenesis, DNA synthesis, cell migration, immune responses, and apoptosis. Thus, gefitinib showed highly promising chemopreventive and chemotherapeutic activity in this mouse model of lung carcinogenesis.

Yang, F., P. Shi, et al. (2006). "Recombinant adenoviruses expressing TRAIL demonstrate antitumor effects on non-small cell lung cancer (NSCLC)." Med Oncol **23**(2): 191-204.

INTRODUCTION: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in a variety of malignant cells, but not in normal cells. This preferential toxicity to the abnormal cells renders TRAIL potentially a very powerful therapeutic weapon against cancer. However, a requirement for large quantities of TRAIL to suppress tumor growth in vivo is one of the major factors that has hindered it from being widely applied clinically. To overcome this, we constructed a replication-deficient adenovirus that carries a human full-length TRAIL gene (Ad-TRAIL) and tested its efficacy against a lung cancer model system in comparison to that of the recombinant soluble TRAIL protein. METHODS: To investigate the antitumor activity and therapeutic value of the Ad-TRAIL on the non-small cell lung cancer (NSCLC), four NSCLC cell lines, namely, YTMLC, GLC, A549, and H460 cells, were used. TRAIL protein expression was determined by Western blotting and flow cytometry. Cell viability was analyzed by proliferation assay, and DNA ladder and cell-cycle analysis were used to identify apoptosis. To further evaluate the effect of Ad-TRAIL in vivo, YTMLC cells were inoculated to the subcutis of nude mice. The Ad-TRAIL was subsequently administered into the established tumors. Tumor growth and the TRAIL toxicity were evaluated after treatment. RESULTS: YTMLC cells infected with Ad-TRAIL showed decreased cell viability and a higher percentage of apoptosis. Similar, Ad-TRAIL treatment also significantly suppressed tumor growth in vivo. CONCLUSIONS: TRAIL gene therapy provides a promising therapy for the treatment of NSCLC.

Yang, Y., T. Ikezoe, et al. (2004). "Proteasome inhibitor PS-341 induces growth arrest and apoptosis of non-small cell lung cancer cells via the JNK/c-Jun/AP-1 signaling." Cancer Sci **95**(2): 176-80.

Proteasome inhibitor PS-341 induces growth arrest and apoptosis of multiple myeloma (MM) cells via inactivation of NF-kappaB in vitro and has afforded some objective responses in individuals with relapsed, refractory MM. However, the activity of PS-341 against non-hematological malignancies remains to be fully elucidated. In this study, we found that PS-341 induced growth arrest and apoptosis of NCI-H520 and -H460 non-small cell lung cancer (NSCLC) cells in conjunction with markedly up-regulated levels of p21(waf1) and p53, and down-regulation of bcl-2 protein in these cells. Also, PS-341 caused phosphorylation of c-Jun NH(2)-terminal kinase (JNK) and c-Jun, and enhanced AP-1/DNA binding activities in these cells as measured by western blotting and enzyme-linked immunosorbent assay (ELISA), respectively. Interestingly, when the JNK/c-Jun/AP-1 signal pathway was disrupted by the JNK inhibitor SP600125, the ability of PS-341 to inhibit the growth of NSCLC cells and to up-regulate the levels of p21(waf1) in these cells was blunted, but the expression of p53 was sustained at a high level, suggesting that the JNK/c-Jun/AP-1 signal pathway might mediate the anti-lung cancer effects of PS-341, with p21(waf1) playing the central role. Thus, PS-341 might be useful for the treatment of individuals with NSCLC.

Yano, S., H. Nokihara, et al. (2003). "Multifunctional interleukin-1beta promotes metastasis of human lung cancer cells in SCID mice via enhanced expression of adhesion-, invasion- and angiogenesis-related molecules." Cancer Sci **94**(3): 244-52.

We examined whether interleukin-1 (IL-1), a multifunctional proinflammatory cytokine, progresses or regresses metastasis of lung cancer. Exogenous IL-1beta enhanced expression of various cytokines (IL-6, IL-8, and vascular endothelial growth factor (VEGF)) and intracellular adhesion molecule-1 (ICAM-1) by A549, PC14, RERF-LC-AI, and SBC-3 cells expressing IL-1 receptors. A549 cells transduced with human IL-1beta-gene with the growth-hormone signaling-peptide sequence (A549/IL-1beta) secreted a large amount of IL-1beta protein. Overexpression of IL-1beta resulted in augmentation of expression of the cytokines, ICAM-1, and matrix metalloproteinase-2 (MMP-2). A549/IL-1beta cells intravenously inoculated into severe combined immunodeficiency (SCID) mice distributed to the lung more efficiently and developed lung metastasis much more rapidly than did control A549 cells. Treatment of SCID mice with anti-IL-1beta antibody inhibited formation of lung metastasis by A549/IL-1beta cells. Moreover, A549/IL-1beta cells inoculated in the subcutis grew more rapidly, without necrosis, than did control A549 cells, which produced smaller tumors with central necrosis, suggesting involvement of angiogenesis in addition to enhanced binding in the high metastatic potential of A549/IL-1beta cells. Histological analyses showed that more host-cell infiltration, fewer apoptotic cells, more vascularization, and higher MMP activity were observed in tumors derived from A549/IL-1beta cells, compared with tumors derived from control A549 cells. These findings suggest that IL-1beta facilitates metastasis of lung cancer via promoting multiple events, including adhesion, invasion and angiogenesis.

Yoshida, K., Y. Yatabe, et al. (2007). "Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer." J Thorac Oncol **2**(1): 22-8.

INTRODUCTION: We evaluated the efficacy of gefitinib monotherapy prospectively in patients with advanced or pretreated non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutations. METHODS: Patients with NSCLC were examined for EGFR exon 19 deletion mutations by fragment analysis and for EGFR L858R point mutations by the Cycleave polymerase chain reaction technique. EGFR mutation-positive patients with locally advanced, metastatic, or recurrent/refractory NSCLC that was not curable with surgery or thoracic radiotherapy were candidates for gefitinib treatment administered at 250 mg/day until disease progression. RESULTS: Mutations of the EGFR gene were detected in 27 (41%) of 66 patients. Ten had exon 19 deletion, and 17 had L858R. Twenty-one patients harboring EGFR mutations were treated with gefitinib and were considered assessable for responses and adverse events. Nineteen patients with EGFR mutations achieved objective responses (three complete responses and 16 partial responses), resulting in an overall response rate of 90.5% (95% confidence interval, 69.6%-98.8%). The median progression-free survival was 7.7 months (95% confidence interval, 6.0 mo to not reached). The median overall survival has not been reached. Common adverse events were skin toxicity, diarrhea, and elevated aminotransferases, but no pulmonary toxicity was observed. CONCLUSIONS: Detection of common EGFR mutations seems to be useful for selecting patients with NSCLC who would likely benefit from gefitinib monotherapy.

Yoshimoto, A., K. Inuzuka, et al. (2007). "Remarkable effect of gefitinib retreatment in a patient with nonsmall cell lung cancer who had a complete response to initial gefitinib." Am J Med Sci **333**(4): 221-5.

Gefitinib is an orally active epidermal growth factor receptor tyrosine kinase inhibitor, and it shows favorable antitumor activity against chemorefractory nonsmall cell lung cancer (NSCLC). However, patients with NSCLC have few treatment options available if they are refractory to gefitinib. We describe a 49-year-old patient with NSCLC who had a complete response to initial gefitinib that lasted for 12 months. The tumor relapsed, and the patient received cytotoxic chemotherapy. However, despite chemotherapy, the patient had radiographic progression of lung metastases and we commenced retreatment with gefitinib, showing a remarkable effect. Epidermal growth factor receptor (EGFR) gene analysis showed deletion mutations in codon 745-750 in exon 19 and EGFR gene amplification. Our case shows that after retreatment with gefitinib, patients may show a remarkable response if they showed a remarkable response to initial gefitinib administration and if a certain time has elapsed since the previous gefitinib treatment.

Yu, J., W. Yue, et al. (2006). "PUMA sensitizes lung cancer cells to chemotherapeutic agents and irradiation." Clin Cancer Res **12**(9): 2928-36.

PURPOSE: Lung cancer, the leading cause of cancer mortality worldwide, is often diagnosed at late stages and responds poorly to conventional therapies, including chemotherapy and irradiation. A great majority of lung tumors are defective in the p53 pathway, which plays an important role in regulating apoptotic response to anticancer agents. PUMA was recently identified as an essential mediator of DNA damage-induced and p53-dependent apoptosis. In this study, we investigated whether the regulation of PUMA by anticancer agents is abrogated in lung cancer cells and whether PUMA expression suppresses growth of lung cancer cells and/or sensitizes lung cancer cells to chemotherapeutic agents and irradiation through induction of apoptosis. EXPERIMENTAL DESIGNS: The expression of PUMA was examined in lung cancer cells with different p53 status treated with chemotherapeutic agents. An adenovirus expressing PUMA (Ad-PUMA), alone or in combination with chemotherapeutic agents or gamma-irradiation, was used to treat lung cancer cells. The growth inhibitory and apoptotic effects of PUMA in vitro and in vivo were examined. The mechanisms of PUMA-mediated growth suppression and apoptosis were investigated through analysis of caspase activation and release of mitochondrial apoptogenic proteins. The cytotoxicities of PUMA on cancer and normal/nontransformed cells were compared. The efficacy of PUMA and p53 in suppressing the growth of lung cancer cells was also compared. RESULTS: We showed that the induction of PUMA by chemotherapeutic agents is abolished in p53-deficient lung cancer cells. PUMA expression resulted in potent growth suppression of lung cancer cells and suppressed xenograft tumor growth in vivo through induction of apoptosis. Low dose of Ad-PUMA significantly sensitized lung cancer cells to chemotherapeutic agents and gamma-irradiation through induction of apoptosis. The effects of PUMA are mediated by enhanced caspase activation and release of cytochrome c and apoptosis-inducing factor into the cytosol. Furthermore, PUMA seems to be selectively toxic to cancer cells and more efficient than p53 in suppressing lung cancer cell growth.

CONCLUSIONS: Our findings indicate that PUMA is an important modulator of therapeutic responses of lung cancer cells and is potentially useful as a sensitizer in lung cancer therapy.

Zhang, P., J. Wang, et al. (2004). "CHK2 kinase expression is down-regulated due to promoter methylation in non-small cell lung cancer." Mol Cancer **3**: 14.

BACKGROUND: CHK2 kinase is a tumor suppressor that plays important role in DNA damage signaling, cell cycle regulation and DNA damage induced apoptosis. CHK2 kinase expression was known to be ubiquitous in mammalian cells. CHK2-/- cells were remarkably resistant to DNA damage induced apoptosis, mimicking the clinical behavior of non-small cell lung cancer to conventional chemo and radiation therapy. RESULT: We reported that the CHK2 expression is diminished or absent in both non-small cell lung cancer (NSCLC) cell lines and clinical lung cancer tumor specimens. The absent CHK2 expression in NSCLC was due to hypermethylation of the CHK2 gene promoter, preventing from binding of a transcriptional factor, leading to silence of the CHK2 gene transcription. CONCLUSION: Since the CHK2 null mice showed a remarkable radioresistance, which bear significant similarity to clinical behavior of NSCLC, down-regulation of CHK2 kinase expression by CHK2 gene silencing and methylation in non-small cell lung cancer suggest a critical role of CHK2 kinase in DNA damage induced apoptosis and a novel mechanism of the resistance of NSCLC to DNA damage based therapy.

Zhang, W., L. P. Stabile, et al. (2006). "Mutation and polymorphism in the EGFR-TK domain associated with lung cancer." J Thorac Oncol **1**(7): 635-47.

BACKGROUND: The epidermal growth factor receptor (EGFR) is involved in the development and progression of lung cancer. Somatic EGFR mutations are predictors of response to treatment with EGFR tyrosine kinase (TK) inhibitors (TKIs) for lung cancer, especially among never smokers. EGFR mutations may occur independently of other genetic alterations. METHODS: The authors sequenced the EGFR-TK domain and the K-ras and p53 genes from lung tumor tissues from 44 never smokers and 46 smokers. A case-control study also was conducted to examine the relationship between an EGFR single nucleotide polymorphism in the TK domain and the lung cancer through a multivariate logistic regression analysis. In addition, the authors compared cell growth kinetics, EGFR-TKI sensitivity by MTT, and activation of signaling molecules by immunoblot in lung cancer cell lines with and without EGFR-TK mutations. RESULTS: EGFR-TK mutations were more frequently observed in never smokers (25%) than in smokers (2.2%) ($p = 0.001$). Excluding cases with a K-ras mutation, the frequency of EGFR-TK domain mutation was still significantly higher in never smokers than in smokers, 26.2% versus 4.5% ($p = 0.046$). EGFR-TK mutations and K-ras mutations ($p = 0.015$), and p53 and K-ras mutations ($p = 0.015$) were mutually exclusive, but p53 and EGFR-TK mutations were not ($p = 1.00$). During sequencing of the EGFR-TK domain in tumors, an EGFR polymorphism (G2607A) was identified. The genotype AA and AA + AG occurred at a significantly higher frequency in lung cancer cases ($n = 122$) when compared with controls ($n = 147$) (odds ratio, 3.39 and 2.67; 95% confidence interval, 1.41-8.17 and 1.17-6.08, $p = 0.006$ and $p = 0.02$, respectively). This polymorphism was found independently of EGFR-TK mutations in lung cancer cases, indicating that it does not predispose to mutations. In vitro, lung cancer cell lines with EGFR-TK mutations also did not contain K-ras mutations and displayed a lower growth rate (50%, $p = 0.013$) than EGFR-TK wild-type cell lines. EGFR-TK mutant cell lines were more sensitive to both gefitinib and erlotinib, although relative sensitivity to erlotinib compared with wild-type was less pronounced than for gefitinib. Cell lines with a lower growth rate also expressed higher levels of E-cadherin than faster growing cell lines. CONCLUSIONS: EGFR-TK mutation frequency is high in never-smoking lung cancer patients and is exclusive of mutation in K-ras but not p53. In addition to somatic EGFR-TK mutations that arise in lung tumors, germline variation in the EGFR-TK domain might also be associated with an increased risk of lung cancer. Somatic EGFR-TK mutations alter cell biology and response to EGFR-TKIs and may be mutation specific.

Zhang, X. and A. Chang (2007). "Somatic mutations of the epidermal growth factor receptor and non-small-cell lung cancer." J Med Genet **44**(3): 166-72.

Frequent overexpression of epidermal growth factor receptor (EGFR) in non-small-cell lung cancer (NSCLC) makes EGFR a new therapeutic target. Two specific EGFR tyrosine kinase inhibitors, gefitinib (ZD1839, Iressa) and erlotinib (OSI-774, Tarceva), have been developed and approved by the US Food and Drug Administration for second-line and third-line treatment of advanced NSCLC. Clinical trials have shown considerable variability in the response rate between different patients with NSCLC, which led to the discovery of somatic EGFR-activating mutations. This brief review summarises the discovery and functional consequences of the mutations, their clinicopathological features and significant

implications in the treatment and prognosis of NSCLC.

Zhang, X., R. M. Cheung, et al. (2005). "Radiotherapy sensitization by tumor-specific TRAIL gene targeting improves survival of mice bearing human non-small cell lung cancer." Clin Cancer Res **11**(18): 6657-68.

PURPOSE: To sensitize non-small cell lung cancer (NSCLC) to radiotherapy by tumor-specific delivery of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene. EXPERIMENTAL DESIGN: The TRAIL was delivered to human NSCLC cell lines and normal human bronchial epithelial cells by the replication-defective adenoviral vector Ad/TRAIL-F/RGD using a tumor-specific human telomerase reverse transcriptase promoter. Cancer growth was studied using 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt and clonogenic assays. Activation of the apoptosis pathway was analyzed in a Western blot and sub-G(1) DNA accumulation. A xenograft mouse lung cancer model was treated by intratumoral injections of Ad/TRAIL-F/RGD and local radiotherapy; the other groups received one of these treatments alone or a control agent. Apoptosis and TRAIL expression in tumors were also analyzed. RESULTS: Ad/TRAIL-F/RGD specifically targets human NSCLC cells without significant effect in normal human bronchial epithelial cells. The combination of Ad/TRAIL-F/RGD and radiotherapy significantly improved cell-killing effect in all NSCLC cell lines tested ($P < 0.05$). Expression of TRAIL showed a dose-dependent relationship with Ad/TRAIL-F/RGD, and radiation seemed to increase TRAIL expression. Activation of the apoptosis by TRAIL and radiation was shown by activation of caspase-9, caspase-8, caspase-3, and poly(ADP-ribose) polymerase and increased DNA sub-G(1) accumulation. The combination of TRAIL and radiotherapy significantly increased apoptosis in vivo, inhibited tumor growth, and prolonged mean survival in mice bearing human NSCLC to 43.7 days compared with 23.7 days (TRAIL only) and 16.5 days (radiotherapy only; $P < 0.05$). CONCLUSIONS: The combination of Ad/TRAIL-F/RGD and radiotherapy significantly improved therapeutic efficacy in suppressing NSCLC tumor growth and prolonging survival. Ad/TRAIL-F/RGD may improve the therapeutic ratio of radiotherapy in NSCLC.

Zhang, X. T., L. Y. Li, et al. (2005). "The EGFR mutation and its correlation with response of gefitinib in previously treated Chinese patients with advanced non-small-cell lung cancer." Ann Oncol **16**(8): 1334-42.

BACKGROUND: The aim of the study was to evaluate the efficacy of gefitinib and the epidermal growth factor receptor (EGFR) mutation to gefitinib response in a series of Chinese patients with pretreated advanced non-small-cell lung cancer (NSCLC). METHODS: A total of 98 patients who had failed at least one platinum-based regimen received gefitinib 250 mg once daily. The mutation analysis of the EGFR kinase domain was performed for 30 patients using paraffin-embedded tumor tissue. RESULTS: The response rate was 31.6% and the disease control rate was 67.3%. Objective response was correlated with adenocarcinoma, female gender and non-smokers. Median progress free survival (PFS) was 7.0 months, median overall survival (OS) was 12.0 months and 1-year survival was 53.1%. The median PFS and OS were improved among patients with adenocarcinoma, gefitinib responders and non-smokers. Active gene mutation was detected in 12 patients. Mutation rates were higher among gefitinib responders, non-smokers, patients with adenocarcinoma and female patients. OS was longer for patients with gene mutation than for patients without mutation. CONCLUSION: Gefitinib demonstrated significant antitumor activity with a favorable toxicity profile for pretreated Chinese patients with advanced NSCLC. The active mutation of the EGFR kinase domain was strongly associated with response to gefitinib and prolonged overall survival.

Zhang, Z., G. Jiang, et al. (2006). "Knockdown of mutant K-ras expression by adenovirus-mediated siRNA inhibits the in vitro and in vivo growth of lung cancer cells." Cancer Biol Ther **5**(11): 1481-6.

The ras mutation, which is observed in 20-30% of human nonsmall cell lung cancers (NSCLCs), is one of common genetic alterations and has been proposed to be a prognostic factor in lung cancer. Oncogene ras appears to be essential for tumor progression and maintenance. Several therapeutic agents have been developed to inhibit ras, such as FTIs and antisense oligonucleotides. A new tool for blocking oncogenes in cancer cells has emerged with the discovery that RNA interference can specifically silence expression of endogenous human genes. In the current study, we used small interfering RNA (siRNA) directed against mutant K-ras to determine the anti-tumor effects of decreasing the levels of this protein in lung cancer cell lines. Adenovirus-mediated siRNA (AdH1/siK-ras(V12)) against K-ras(V12) markedly decreased K-ras(V12) gene expression and inhibited cellular proliferation of NSCLC H441 cells that express the relevant mutation (K-ras codon 12 GGT --> GTT), but produced minimal growth inhibition on NSCLC H1650 cells that lack the relevant mutation.

Pretreatment with AdH1/siK-ras(V12) completely abrogated subcutaneous engraftment of H441 cells, as compared with a 100% tumor take in animals that received control vector-treated tumor cells. The in vivo effect of AdH1/siK-ras(V12) treatment was further examined by intratumoral injections after tumor induction. Pre-existing tumor growth was reduced by 45% by a single intratumoral injection. Three or five repeat injections resulted in complete tumor regression in eight of ten nude mice. Further, 23.12% of AdH1/siK-ras(V12) treated H441 cells underwent apoptosis, as compared with 6.13%, and 8.27% in untreated and control vector-treated cells, respectively. These results indicate that adenovirus-mediated siRNA can specifically and efficiently target factors whose expression is altered in malignancy and may have the potential as a therapeutic modality to treat human lung cancer.

Zhong, S., C. R. Fields, et al. (2007). "Pharmacologic inhibition of epigenetic modifications, coupled with gene expression profiling, reveals novel targets of aberrant DNA methylation and histone deacetylation in lung cancer." Oncogene **26**(18): 2621-34.

Lung cancer is the leading cause of cancer-related deaths in the United States due, in large part, to the lack of early detection methods. Lung cancer arises from a complex series of genetic and epigenetic changes leading to uncontrolled cell growth and metastasis. Unlike genetic changes, epigenetic changes, such as DNA methylation and histone acetylation, are reversible with currently available pharmaceuticals and are early events in lung tumorigenesis detectable by non-invasive methods. In order to better understand how epigenetic changes contribute to lung cancer, and to identify new disease biomarkers, we combined pharmacologic inhibition of DNA methylation and histone deacetylation in non-small cell lung cancer (NSCLC) cell lines, with genome-wide expression profiling. Of the more than 200 genes upregulated by these treatments, three of these, neuronatin, metallothionein 3 and cystatin E/M, were frequently hypermethylated and transcriptionally downregulated in NSCLC cell lines and tumors. Interestingly, four other genes, cylindromatosis, CD9, activating transcription factor 3 and oxytocin receptor, were dominantly regulated by histone deacetylation and were also frequently downregulated in lung tumors. The majority of these genes also suppressed NSCLC growth in culture when ectopically expressed. This study therefore reveals new putative NSCLC growth regulatory genes and epigenetic disease biomarkers that may enhance early detection strategies and serve as therapeutic targets.

Zhou, C., J. Ni, et al. (2006). "Rapid detection of epidermal growth factor receptor mutations in non-small cell lung cancer using real-time polymerase chain reaction with TaqMan-MGB probes." Cancer J **12**(1): 33-9.

We investigated somatic mutations of the epidermal growth factor receptor gene in non-small cell lung cancer tumor tissue and their detection using real-time polymerase chain reaction with TaqMan-MGB probes. METHODS: The DNA was extracted from surgically resected non-small cell lung cancer tumor specimens. Genes encoding for epidermal growth factor receptor tyrosine (exons 18, 19, and 21) were amplified by nested polymerase chain reaction, sequenced, and analyzed by chromatograms with manual review. TaqMan-MGB probes were designed to detect the epidermal growth factor receptor gene mutations in the tumor tissues using real-time polymerase chain reaction. RESULTS: Somatic point mutations and deletions were identified in the tyrosine kinase domain of the epidermal growth factor receptor gene in 21 of 80 non-small cell lung cancer patients, including 13 patients with deletion mutations occurring in exon 19 and 8 patients with point mutations occurring in codon 858 (exon 21). The results from real-time polymerase chain reaction with TaqMan-MGB probes were completely consistent with sequencing outcomes. Both the sensitivity and specificity for detecting the epidermal growth factor receptor gene mutations using real-time polymerase chain reaction with TaqMan-MGB probes were 100%. The mutation incidence was significantly higher in female patients, nonsmokers, and patients with adenocarcinoma than in male patients, smokers, and those with nonadenocarcinomas ($P < 0.05$). The mutations were not related to patient's age or tumor nodal metastasis staging. CONCLUSIONS: Somatic mutations of the epidermal growth factor receptor gene that develop in non-small cell lung cancer patients are more common in female patients, nonsmokers, and patients with adenocarcinoma. Real-time polymerase chain reaction using TaqMan-MGB probes is effective, simple, and fast in the detection of epidermal growth factor receptor gene mutations.

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9/12/2012