9025 POSTER

Expression of cyclooxygenase-2 in cytological material from patients with lung cancer

A. Koch[1], S. Sörenson[2], H. Fohlin[3], B. Gustafsson[4]. [1]*Allergy Center and Department of Pulmonary Medicine, University Hospital and Department of Medical and Health Sciences Linköping University, Linkoping, Sweden;* [2]*Department of Medicine County Hospital Jönköping, Department of Medical and Health Sciences Linköping University, Linköping, Sweden;* [3]*Oncologic Centre, University Hospital, Linköping, Sweden;* [4]*Department of Pathology and Cytology, University Hospital, Linköping, Sweden*

Background: Cyclooxygenase-2 (COX-2)-expression may be predictive for the effect of celecoxib in patients with advanced non-small cell lung cancer (NSCLC). In previous studies, COX-2-expression has almost exclusively been evaluated with immunohistochemical methods performed on histology sections of tissue biopsies. However, in clinical practice, lung cancer is often diagnosed with cytological techniques only. Methodology and results from analysis of COX-2-expression in cytological material from lung cancer patients by immunocytochemistry have, to our knowledge, not been described previously.
Material and Methods: Fifty-three patients with lung cancer were prospectively examined. Material was obtained from routine diagnostic transbronchial fine-needle aspirations or transthoracic needle aspiration. Slides with obvious tumour cells were selected, fixed in 4% paraformaldehyde and immunostained with monoclonal antibody mouse-anti-human COX-2. An experienced cytopathologist evaluated the slides as well as routinely stained parallel slides. Percentage stained tumour cells (<1%, 1−10%, 11−50%, >50%) and intensity of staining (none, weak, strong) were estimated. Clinical data were collected from patient records.
Results: There were 32 men and 21 women with median age 68 years (range 43−87). Eighty-nine percent had NSCLC. Preparation and staining with the methods established at our laboratory were easy to perform. Quality and readability of the slides were generally good. Tumour cells, singly and in clusters, were easily discriminated from benign cells. The percentage COX-2-stained cells and the intensity of staining varied widely between and within the different cases. The proportion of positively stained tumour cells was as follows: <1%: 20 pts., 1−10%: 7 pts., 11−50%: 17 pts., more than 50%: 9 pts. In 17 cases, groups of cells with different intensity of COX-2-staining were found in the same slide. There were no significant differences in survival when grouping the cases according to percentage of COX-2-expression.
Conclusions: Immunocytochemical analysis of COX-2-expression is technically easy to perform with routine diagnostic procedures resulting in easily readable, high quality slides. There is a great variation in the proportion of COX-2-positive cells from case to case as well as in the intensity of staining between individual cells in many single cases.

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The prognostic significance of [18F]flurodeoxyglucose uptake by positron emission tomography in advanced non small cell lung cancer

H.M. Ryoo[1], K.U. Park[2], S.H. Bae[1]. [1]*Daegu Catholic University Medical Center, Division of Hemato-oncology, Daegu, Korea;* [2]*Keimyung Univ Dongsan Hospital, Division of Hemato-oncology, Daegu, Korea*

Background: Lung cancer is the leading cause of cancer-related death in Korea. Non small cell lung cancer(NSCLC) comprises 80−85% of lung cancer. Positron emission tomography with [18F]fluorodeoxyglucose(FDG-PET) shows various levels of FDG uptake for patients with NSCLC. The aims of this study were to determine whether the standardized uptake value (SUV) of FDG uptake by PET could be a prognostic factor for advanced NSCLC.
Method: FDG-PET was performed for 59 patients with stage IIIb and IV non small cell lung cancer. The SUV was calculated for each patient. Overall survival(OS), progression free survival(PFS) were calculated by the Kaplan- Meier method and evaluated with the log-rank test. The prognostic significance was assessed by univariate and multivatiate analysis.
Results: A cutoff of 7 for the SUV showed the best criminative value. In a univariate analysis, performance status($p = 0.02$) and SUV($p = 0.03$) were the significant predictors of OS. The patients with low SUVs($\leqslant 7$) showed significantly better PFS than those with high SUVs(>7, $p = 0.04$). A multivariate Cox analysis identified performance status and the SUV as important for the prognosis.
Conclusion: These results suggest that SUV was the significant prognostic factor among the patients with advanced non small cell lung cancer.

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Patient-derived tumourgrafts of non-small cell lung cancer (NSCLC) as models for the identification of predictive biomarkers for classical and targeted therapies

I. Fichtner[1], J. Rolff[1], J. Merk[2], R. Soong[3], A. Lamich[1], M. Rivera[1], M. Becker[4], J. Hoffmann[5]. [1]*Max Delbrück Center for Molecular Med, Experimental Pharmacology, Berlin, Germany;* [2]*Evangelische Lungenklinik, Chirurgie, Berlin, Germany;* [3]*National University of Singapore, Oncology Research Institute, Singapore, Singapore;* [4]*Experimental Pharmacology and Oncology GmbH, Molecular Biology, Berlin, Germany;* [5]*Experimental Pharmacology and Oncology GmbH, Pharmacology, Berlin, Germany*

Background: With the newly developed tyrosine kinase inhibitors drugs are available for the targeted treatment of patients with NSCLC. But clinical trials revealed no clear correlation between the EGFR expression and the response to targeted therapy. Several aspects concerning the individual prediction of response (mutations, expression levels) have been investigated but did so far not result in the acceptance of a routine biomarker for clinical use. Therefore, we intended to use a novel xenotransplantation system for the investigation of clinically relevant biomarkers.
Material and Methods: We have developed a series of novel lung cancer tumorgrafts. Fresh tumor material of patients with NSCLC was subcutaneously transplanted to immunodeficient mice shortly after removal. 25 passagable models could be generated and were used for the definition of predictive biomarkers and concerning the responsiveness to classical (Paclitaxel, Gemcitabine, Carboplatin, Vinorelbine, Etoposide) or targeted (Cetuximab, Erlotinib) therapies.
Results: It was demonstrated that the early murine passages correlated with the original tumor regarding histology, the expression of the surface proteins and in gene profiling (Affymetrix).
12/25 tumorgrafts were sensitive towards Cetuximab and 6/25 towards Erlotinib. None of the tumorgrafts showed functional mutations in the EGFR gene. 5/25 models with K-ras mutations were found among the xenografts; four of these tumors were resistant towards Erlotinib. In 12/25 different models mutations in the p53 gene could be located. All xenografts are Met wt and only two revealed PI3K mutations. None of these mutations was clearly correlated with a specific response towards anti-EGFR therapies. 23/25 tumors were positive for E-cadherin and 7/25 for Vimentin suggesting that the potential for epidermal-mesenchymal transition is not directly correlated with any therapeutic response. But in a few sensitive tumors a translocation of E-cadherin into the nucleus was found after Erlotinib treatment. The expression of EGFR ligands like TGFalpha and epiregulin was weakly correlated with the response to Erlotinib or Cetuximab.
Conclusions: In summary, we have established a panel of well characterized NSCLC tumorgrafts. These models better represent the heterogeneity of lung cancer, correlate with the clinical situation and are able to identify biomarkers and their regulation after therapeutic interventions both at genetic and at protein level.

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Genetic polymorphisms of the endothelial nitric oxide synthase gene correlate with overall survival in advanced non-small-cell lung cancer treated with platinum-based doublet chemotherapy

S. Fujita[1], K. Masago[2], R. Kaji[1], A. Hata[1], S. Nanjo[1], K. Otsuka[1], T. Mio[2], M. Mishima[2], N. Katakami[1]. [1]*Institute of Biomedical Research and Innovation, Division of Integrated Oncology, Kobe, Japan;* [2]*Kyoto University Hospital, Department of Respiratory Medicine, Kyoto, Japan*

Background: Nitric oxide (NO) is a small free radical that is involved in carcinogenesis. Endothelial NO, synthesized from L-arginine by endothelial NO synthase (eNOS), inhibits apoptosis and promotes angiogenesis, tumor cell proliferation and metastasis. The aim of this study was to evaluate the influence of eNOS gene polymorphisms on the prognosis of patients with advanced-stage non-small-cell lung cancer (NSCLC).
Patients and Methods: Unresectable, chemotherapy naïve stage III or IV NSCLC patients treated with a standard platinum-containing doublet regimen were analyzed. All individuals were genotyped for a single-nucleotide polymorphism (SNP), 894 G to T in exon 7, and a variable number of tandem repeats (VNTR) polymorphism in intron 4, a rare smaller allele (a) and a common larger allele (b), to elucidate a potential association between these polymorphisms and clinical outcome.
Results: From July 2004 to July 2007, a total of 108 patients (male/female: 66/42, PS 0/1 = 23/85, Stage IIIA/IIIB/IV = 6/30/72), aged 29−77 (Median: 63) years, were consecutively enrolled in this study. Definite thoracic radiotherapy was administered in 20 (18.5%) patients. The median survival was 26.8 months. Using Kaplan-Meier estimates to calculate 5-year probabilities of follow-up, we could show that overall survival

was significantly increased in patients who were VNTR a-allele carriers, compared to VNTR b/b patients (P = 0.015). In multivariate Cox proportional hazard analysis, the VNTR polymorphism was an independent prognostic factor for survival. Homozygous b/b patients were at higher risk for death (HR, 2.22; P = 0.013) compared with a-allele carriers.
Conclusions: The results support the role of the VNTR polymorphism in intron 4 as a marker for survival in patients with advanced-stage NSCLC who were fit for standard chemotherapy. Updated data will be reported.

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Prognostic value of immunohistochemical stain pattern for carcinoembryonic antigen in patients with completely resected pathological stage I non-small cell lung cancer

H. Suzuki[1], H. Matsuguma[1], S. Igarashi[2], R. Nakahara[1], N. Ohata[1], T. Kasai[3], Y. Kmiyama[3], M. Mori[3], T. Kodama[3], K. Yokoi[4]. *[1]Tochigi Cancer Center, Thoracic Surgery, Utsunomiya, Japan; [2]Tochigi Cancer Center, Pathology, Utsunomiya, Japan; [3]Tochigi Cancer Center, Thoracic Disease, Utsunomiya, Japan; [4]Nagoya University Graduate School of Medicine, Thoracic Surgery, Nagoya, Japan*

Background: Surgery alone remains the standard therapy for patients with stage I NSCLC based on the recent results of randomized control trials. There is, however, a subgroup of patients with stage I disease who have a poor prognosis, for whom adjuvant chemotherapy can be as effective as that for patients with more advanced disease. Preoperative serum carcinoembryonic antigen (CEA) levels have been reported to be an independent prognostic factor for stage I NSCLC. Nevertheless, adjuvant therapy is not performed based on the serum CEA levels, because serum CEA levels can be influenced by smoking or other lung conditions. Therefore, a more definitive indicator is considered necessary. We hypothesized that immunohistochemical (IHC) CEA expression would be a more reliable and effective prognostic marker than serum CEA levels.
Material and Methods: Between 1986 and 2000, 333 patients who underwent complete resection at our hospital were diagnosed as having stage I lung cancer. Immunohistochemical staining with the antibody for CEA was carried out on paraffin embedded sections of those tumors using the avidin-biotin-peroxidase complex method. Staining patterns were classified into three patterns: Type1: CEA immunoreactivity was negative or demonstrated only the cell surface; Type2: CEA immunoreactivity was distributed in the cytoplasm; Type3: CEA immunoreactivity was demonstrated both at the cell surface and in the cytoplasm. Preoperative serum CEA levels and other clinicopathological factors were also investigated by univariate and multivariate analysis.
Results: The below table shows the number of patients, 5-year survival rates, and serum CEA levels according to the CEA IHC pattern. The CEA IHC pattern was significantly associated with serum CEA levels (P < 0.0001). Univariate analysis revealed age, sex, smoking history, tumor size, histology, lymphatic invasion, vascular invasion, pleural invasion, serum CEA levels, and CEA IHC pattern was significant prognostic factors. With regard to histology, univariate analysis revealed that the CEA IHC pattern was a significant prognostic factor only in patients with adenocarcinoma, however, not in non-adenocarcinoma. Multivariate analysis conducted only in patients with adenocarcinoma disclosed that CEA IHC type 3, vascular invasion, and older age were independent adverse prognostic factors.

CEA IHC pattern	All patients	5yOS	Ad	5yOS	Non-Ad	5yOS	High serum CEA level
type1	140	82.9	102	90.2	38	63.2	31(22.1%)
type2	169	65.1	107	72.9	62	51.6	69(40.8%)
type3	24	41.7	13	38.5	11	45.5	17(70.8%)
p value		<0.0001		<0.0001		0.8903	

Conclusions: The CEA IHC pattern was a more effective prognostic marker than serum CEA levels for patients with pathological stage I lung adenocarcinoma.

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Human Mena (hMena) and isoforms hMena+11a and hMenadeltaV6, estrogen receptor-beta (ER-B), epidermal growth factor receptor −1 and −2 (EGFR/HER-2) expression as prognostic factors in node-negative Non-Small-Cell Lung Cancer (NSCLC)

E. Bria[1], M. Mottolese[2], I. Sperduti[3], P. Visca[2], B. Antoniani[2], F. Facciolo[4], F. Di Modugno[5], F. Cognetti[1], P. Nisticò[5], M. Milella[1]. *[1]Regina Elena National Cancer Institute, Medical Oncology, Rome, Italy; [2]Regina Elena National Cancer Institute, Pathology, Rome, Italy; [3]Regina Elena National Cancer Institute, Biostatistics-Scient. Dir., Rome, Italy; [4]Regina Elena National Cancer Institute, Thoracic Surgery, Rome, Italy; [5]Regina Elena National Cancer Institute, Immunology Lab, Rome, Italy*

Background: hMena is a cytoskeleton regulatory protein involved in adhesion and cell motility, particularly in response to EGFR activation. In addition, a possible correlation between ER-B and EGFR expression has been recently suggested in NSCLC. We therefore investigated the potential relationship and prognostic value of hMena, hMena^{+11a} and hMena^{deltaV6}, ER-B, EGFR and HER-2 expression in node-negative NSCLC patients (pts) who underwent surgery at our institution.
Methods: hMena (plus isoforms), ER-B (isoforms 1, 2), EGFR and HER-2 expression, analyzed on 2 Tissue Micro Array (TMA) copies, were correlated to disease-free, cancer-specific, and overall survival (DFS/CSS/OS) using a Cox model including sex, stage, age, grading, histology, number of resected nodes (RN). Logistic and generalized linear models were used to evaluate predictors of significant Cox-model variables. Receiver Operative Curve (ROC) analysis identified optimal cut-off values. Internal cross-validation (100 simulations with 80% of the dataset) was accomplished.
Results: 248 pts were gathered (median follow-up 36 months, range 1−96; male/female 71/29%; adeno/other 43/57%; grading G1−2/G3 45/55%; Stage I/II 82/8%; RN ⩽10/>10 34/66%). No significant difference between the 2 TMA copies was found for each factor. Multivariate analysis, is shown in the table:

	DFS		CSS		OS	
	HR (95% CI)	p	HR (95% CI)	P	HR (95% CI)	p
RN	1.84 (1.16, 2.94)	0.01	–	n.s.	1.83 (1.10, 3.05)	0.02
Stage	1.76 (1.00, 3.09)	0.05	2.56 (1.24, 5.28)	0.01	1.98 (1.10, 3.58)	0.02
hMena	1.67 (1.00, 2.81)	0.05	2.34 (1.22, 4.51)	0.01	–	n.s.
hMena^{+11a}	1.85 (1.10, 3.12)	0.02	1.88 (0.93, 3.82)	0.08	1.68 (0.97, 2.91)	0.06
hMena^{deltaV6}	1.58 (0.91, 2.73)	0.10	–	n.s.	1.78 (1.00, 3.20)	0.05
ER-B	–	n.s.	1.01 (1.00, 1.02)	0.07	–	n.s.

Pts with hMena^{+11a} overexpression (cut-off >50 according to ROC analysis) have a significantly better 3-yrs DFS and CSS (69.5% versus 58.9%, log-rank p = 0.03) and a better OS (68% vs 75.4%, p = 0.06). EGFR strongly predicted both hMena isoforms overexpression (p = 0.005, p = 0.03); indeed, when hMena was removed from the multivariate model, EGFR was independent predictor of CSS (p = 0.07). Cross-validation analysis confirmed the prognostic role of hMena and isoforms with a replication rate of 51/72% for DFS/CSS.
Conclusions: hMena, hMena^{+11a} and hMena^{deltaV6} expression is prognostic in early NSCLC undergoing curative surgery. EGFR strongly correlate with hMena status and their prognostic role deserves further investigation.

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Lung cancer in women: the Spanish female-specific database WORLD 07

M. Majem[1], M. Domine[2], P. Lianes[3], F.J. Dorta[4], S. Catot[5], C. Guillen[6], R. De las Peñas[7], C. Vadell[8], M.L. Amador[9], R. Rosell[10]. *[1]Hospital de Sant Pau Barcelona, Medical Oncology, Barcelona, Spain; [2]Fundación Jimenez Diaz, Medical Oncology, Madrid, Spain; [3]C.S. del Maresme, Medical Oncology, Mataró, Spain; [4]Hospital Universitario Nuestra Señora de la Candelaria, Medical Oncology, Sta Cruz de Tenerife, Spain; [5]Fundació Althaia, Medical Oncology, Manresa, Spain; [6]Hospital general Universitario de Elche, Medical Oncology, Elche, Spain; [7]Hospital provincial de Castellón, Medical Oncology, Castellón, Spain; [8]Hospital de Manacor, Medical Oncology, Manacor, Spain; [9]Roche, Medical Oncology, Madrid, Spain; [10]ICO-Hospital Germans Trias i Pujol, Medical Oncology, Badalona, Spain*

Background: Lung cancer is the leading cause of cancer mortality among women in many countries. Gender differences have been reported, most of them based on retrospective analysis.
Materials and Methods: WORLD07 is a prospective, multicenter, epidemiologic female-specific lung cancer database developed by the Spanish Lung Cancer Group. Data on demographics, previous cancer