motility in the sensitive H1395 was reduced in combined EGF/Cetuximab treated samples, compared to the EGF treated alone. Later, qRT-PCR analysis revealed that u-PAR, an invasion related gene was differentially expressed, as the EGF stimulation led to a 3-fold induction of u-PAR mRNA which was brought down to the basic level in Cetuximab/EGF treated samples. Luciferase reporter assays showed that EGF induces u-PAR promoter activity, but not in the Cetuximab-pretreated sample. EMSA helped to identify AP1 as the transcription factor found to have less binding intensity in the Cetuximab/ EGF vs EGF treated samples. Whereas, the other two major transcription factors (Sp1/Sp3/AP2 like, NF-kB) in the u-PAR promoter were found not to be altered in both EGF and Cetuximab/EGF treated samples. Supershift analysis showed the major AP1 family members that bound differentially after EGF stimulation and Cetuximab inhibition are c-Jun and Jun D.
Conclusions: Cetuximab is an efficient inhibitor in terms of migration and invasion of the NSCLC tumor cells. When targeting EGFR with Cetuximab, u-PAR, an invasion related gene is downregulated transcriptionally.

6534 POSTER

Elevated levels of thioredoxin (Trx) in serum correlate with poor outcome in docetaxel (doc)/cisplatin (cis)-treated stage IV non-small-cell lung cancer (NSCLC) patients (pts)

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Background: Chemotherapy causes the production of reactive oxygen species (ROS), which facilitates cancer cell death. Trx protein functions as a ROS scavenger and a negative regulator of apoptosis signal regulating kinase-1 (ASK-1). High levels of Trx are associated with chemoresistance. 14-3-3σ proteins are involved in cell cycle control and protein trafficking.
Methods: Trx ELISA and 14-3-3σ methylation-specific PCR were performed in baseline serum from 107 stage IV NSCLC pts treated with doc/cis.
Results: Median age, 60 (range, 32−79); male, 87 (81.3%). PS: 0, 27 (25.2%); 1, 80 (74.8%). Adenocarcinoma, 46 (43.8%); squamous cell carcinoma, 40 (38.1%); 21 pts had large cell or unspecified histology. Complete response, 1 pt; partial response, 20 pts; overall response rate, 20%. Median Trx level, 97.4 (range, 18.8–763.1). Serum was available for 14-3-3σ methylation analysis in only 88 pts. 14-3-3σ was methylated in 43 pts (48.9%). A significant correlation was observed between 14-3-3σ methylation status and Trx levels (Table). 4 pts with methylated and 17 with unmethylated 14-3-3σ had Trx levels >182.8 (P = 0.003). Median Trx levels were 103.5 in responders and 94.3 in non-responders (P = 0.96). Time to progression (TTP) was 5.6 months (mo) for 27 pts with Trx < 49.6, 4.4 mo for 53 pts with Trx 49.6–182.8, and 3.8 mo for 27 pts with Trx > 182.8 (P = 0.02). In a Cox multivariate analysis, Trx levels emerged as an independent variable for TTP when 14-3-3σ was included in the model. Hazard ratios: 1.3 for PS1 (P = 0.84); 1.05 for 14-3-3σ unmethylated (P = 0.22); 1.4 for Trx 49.6–182.8 and 1.95 for Trx >–182.8 (P = 0.04).
Conclusions: Serum Trx levels can predict TTP in doc/cis-treated pts. The additional role of 14-3-3σ methylation may be more clearly demonstrated in cis/gemcitabine regimens.

14-3-3 σ	Trx Levels		
	⩽49.7	49.7–182.8	>182.8
methylated	11 (25.6%) (47.8%)	28 (65.1%) (63.6%)	4 (9.3%) (19%)
unmethylated	12 (26.7%) (52.2%)	16 (35.6%) (36.4%)	17 (37.8%) (81%)

6535 POSTER

Predictive role of biological markers in NSCLC patients (pts) treated with EGFR tyrosine kinase inhibitors (TKIs): a metanalysis of randomized trials

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The magnitude of survival benefit of TKIs in pts with advanced non small lung cancer (NSCLC) is small. However, a growing body of evidence supports a greater survival benefit of TKIs in pts with EGFR mutations, EGFR amplification and or EGFR overexpression. Furthermore, a negative outcome in those pts with K-ras mutations is reported.
We performed a pooled analysis of randomized phase II and III trials to assess the role of these factors in predicting efficacy of TKIs.
An electronic search focused on all phase II and III trials assessing efficacy of TKIs alone or associated with chemotherapy in NSCLC was performed. Evaluable trials had to report at least a subgroup analysis for EGFR tests. A pooled analysis was accomplished and Hazard Ratio (HR) with 95% confidence interval was derived for each level of analysed factors.
Four trials (ISEL, INTACT, TRIBUTE, BR21) were considered for analysis. Sufficient data were available only for analysis of EGFR mutation, EGFR amplification and EGFR over-expression. Only one trial was evaluable for K-ras. Results are reported in the table.

	No. of pts evaluable	HR	L95	U95	LogRank P value	Interaction P value	HR	L95	U95	LogRank P value	Interaction P value
Mutations											
Negative	389	0.85	0.70	1.02	0.083		0.97	0.78	1.21	0.781	
Positive	58	0.92	0.53	1.58	0.751		0.51	0.26	1.02	0.057	
Overall	447	0.86	0.72	1.02	0.081	**0.796**	0.91	0.74	1.13	0.401	**0.084**
Amplification											
Negative	489	1.03	0.85	1.25	0.748		0.94	0.77	1.14	0.526	
Positive	89	0.63	0.43	0.92	0.016		0.60	0.39	0.92	0.019	
Overall	578	0.93	0.79	1.11	0.434	**0.022**	0.87	0.73	1.04	0.121	**0.061**
Over-expression											
Negative	141	1.08	0.78	1.50	0.632		1.24	0.77	2.01	0.382	
Positive	184	0.72	0.57	0.91	0.007		0.83	0.61	1.12	0.229	
Overall	325	0.83	0.69	1.00	0.054	**0.048**	0.93	0.72	1.20	0.581	**0.168**

Only EGFR amplification (p = 0.022) and EGFR over-expression (p = 0.048) showed a predictive effect on overall survival, whereas no clear evidence was detected in PFS analysis. EGFR mutations don't seem to have a predictive role. These evidences need further confirmation from large prospective randomized trials powered specifically for predictive factors analysis. The clear identification of them may help in implementation of a more effective strategy for the treatment of NSCLC pts and could lead to a more rational use of TKIs.

6536 POSTER

CYP3A5 polymorphism and NSCLC – a role for genetic variation as a protective factor in lung cancer susceptibility

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Background: Lung cancer (LC) is the most common cancer in Europe (381,500 new cases in 2004) and the third in the USA (172,570 new cases in 2005). Smoking is one of the major causes of LC: there are many procarcinogens present in tobacco smoke that, when activated, contribute to the development of this disease. The CYP3A subfamily represents a group of enzymes responsible for the metabolism of many currently used drugs, exogenous carcinogens and endogenous molecules, such as steroids. Two of the major enzymes in this family, CYP3A4 and CYP3A5, activate polycyclic aromatic hydrocarbons, such as benzo[a]pyrene and other procarcinogens present in tobacco smoke. Functional polymorphisms, such as CYP3A5*3 (characterized by an A to G transition and associated with the lack of the CYP3A5 protein), could alter individual susceptibility to LC. The aim of our study was to evaluate the influence of this polymorphism in the development of LC.
Material and Methods: DNA samples were extracted from peripheral blood cells of 711 individuals: 246 patients with non-small cell lung cancer (NSCLC), which included 137 smokers, 49 ex-smokers and 51 non smokers (data was not available for 9 patients) and 465 blood donors. The CYP3A5*3 polymorphism was analysed through PCR-RFLP (SspI). Analysis of data was performed using the computer software SPSS for windows. The odds ratio (OR) and its 95% confidence interval (CI) were

calculated as a measure of the association between CYP3A5*3 genotypes and NSCLC progression.
Results: The frequency of the GG genotype was 79.3% in LC patients and 86% in controls. The frequency of the heterozygous genotype A/G was of 20.3% in patients and 13.3% in controls. Homozygous individuals for the A allele where rare: 0.1% in LC patients and 0.6% in controls. The analysis of the genotypic frequencies of the CYP3A5*3 polymorphism indicates that individuals with GG genotype present a 38% protection for the development NSCLC (P = 0.020; OR = 0.621; 95% CI = 0.415−0.931).
Conclusions: Individual differences in the metabolism of carcinogens may influence the susceptibility to cancer development and behaviour. Our results suggest that that individuals with GG genotype present a lower risk of developing NSCLC than individuals with genotypes carrying the A allele (OR = 0.621). This is probably due to a decreased activation of procarcinogens present in tobacco smoke in result of the lack of CYP3A5 in individuals with genotypes carrying the CYP3A5*3 allele.

6537 POSTER

Selenite mediated cytotoxcicity in human lung cancer and the role of Thioredoxin reductase 1

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Background: The human selenoenzyme thioredoxin reductase 1 (TrxR1) is a very important enzyme for cell growth, differentiation, and the defense against oxidative stress. Several studies have shown that TrxR1 is upregulated in tumor cells and it is a target for many anti-cancer drugs. The regulation of TrxR1 is very complex and involves the expression of different transcript forms of mRNA.
Materials and Methods: We have, by quantitative polymerase chain reaction, investigated the total expression of TrxR1 mRNA and quantified the expression of alternative mRNA forms in five different human lung cancer cell lines. IC50 values for selenite were determined for the different cell lines and compared to the sensitivity towards doxorubicin.
Results: The results indicated an inverse relationship between resistance towards doxorubicin and selenite induced cytotoxicity. In addition, inhibition of TrxR resulted in enhanced selenite cytotoxicity. Selenium treatment resulted in increased expression of almost all TrxR1 mRNA variants while the TrxR protein activity decreased. Total TrxR1 and the less abundant forms were detected in human tissue samples from both squamous and adenocarcinoma from lung, using specific peptide antibodies. Expression of TrxR1_v.2, 3, 5 isoforms and Trx1 in the tumor correlated with degree of differentiation.
Conclusions: Our results show that TrxR1 is involved in selenite mediated cytotoxicity and investigation of alternative transcript variants of TrxR1 could further be a valuable tool in the diagnostics and characterization of tumors.

6538 POSTER

Preclinical studies on the antitumor activity induced by novel modified steroidal alkylating esters of propenoic acid against murine Lewis lung carcinoma (LLC)

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Background: The sensitivity of some neoplasms to hormonal intervention provides a rational basis for utilizing steroid hormones as a biological “platform” for cytotoxic agents in cancer therapy. The purpose of this study is to investigate the relationship between structure activity and antineoplastic effect of in vivo biological system, treated by five newly synthesized modified steroidal derivatives of p-bis(2-chloroethyl)aminophenylpropenoic acid (PK 11–PK 15).
Materials and Methods: The acute toxicity of the compounds was determined fol-lowing a single i.p. injection into C57BL mice in groups of 10 mice/dose. C57BL mice were used for the evaluation of the antitumor activity. Experiments were initiated by implanting the LLC cells. These were injected subcutaneously [0.2 ml tumor brei of 13 (w:v)]. Each treated group consisted of 6 mice and 8 mice comprised the control group, treated with saline only.
Results: The antitumor activity was assessed from the inhibition of tumor size (I) and from the oncostatic parameter (T/C).
Conclusions: The antitumor activity of compound PK 11 is distinctly superior to that of compounds PK 12, PK 14, PK 15, whose activity is marginal. Compound PK 13 is less effective than PK 11. Most likely, the highest effect of compound PK 11 is due to the presence of double bond in the homoazasteroidal nucleus (ring B) and the 3β-(cis) configuration.

Acute toxicity and antitumor activity of PK 11–PK 15 on LLC.

Compound	LD10 (mg/kg)	LD10 dose (mg/kg)	Treatment schedule	Growth inhibition (%)	T/C (%)
Control	–	Saline	–	0	100
PK 11	500	500	Day 1	57	194
PK 12	400	400	Day 1	28	127
PK 13	600	600	Day 1	42	171
PK 14	200	200	Day 1	21	124
PK 15	100	100	Day 1	19	117

6539 POSTER

Malignant mesothelioma of pleura: potentialities of immunocytochemistry

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Considering that surgical treatment is extremely traumatic, pathologist must be sure in the diagnosis. It is very important to use supplementary methods of diagnostics.
An objective of this work was to study potentialities of cytological diagnostics of pleural mesothelioma using immunocytochemistry. The data of 90 patients with malignant mesothelioma of pleura was investigated in Altai Oncological Hospital during 5 years including females 48 (53.3%) and males 42 (46.7%). Immunocytochemistry was used from 65 (72.2%) patients. Specimens were prepared using Centrifuge and Streptavidin-biotin system with a set of markers (11 antibodies).
Epithelioid mesothelioma was diagnosed in 82 cases (91.1%). The sarcomatoid variant of pleural mesothelioma was determined in 2 (2.2%) and biphasic in 6 (6.7%) cases. Both cases of sarcomatoid mesothelioma resemble fibrosarcoma. For differential diagnostics immunocytochemistry was used. The reactions with Cytokeratins (C MNF 116, C AE1/AE3) were most important. Biphasic mesothelioma contains both epithelial and sarcomatoid cells.
The cells of mesothelioma were positive with Keratins (C MNF 116, C AE1/AE3), also positive cytoplasmatic reactions with Vimentin were noticed in all cases of mesothelioma. The cells of tumours were immunonegative with mono- and polyclonal Carcinoembrionin antigen (CEA). Tumour cells had weak reactivity with polyclonal Carcinoembrionin antigen in 3.1% of cases. Immunonegative reaction of mesothelioma cells was noticed with Epithelial antigen (Ber-EP4).
All cases of mesothelioma (100%) showed positive reactions with Methotelial Cell (HBME-1). Calretinin and Thrombomodulin were studied only in 12 cases of mesothelioma (18.5%). Cells of mesothelioma with Calretinin had nuclear and cytoplasmaticstaining.It wasn’t noticed in cells of carcinoma excluding serous papillary of ovarian carcinoma. Metastases of carcinoma had immunonegative reactions with Thrombomodulin. Cells of mesothelioma showed negative reactions with CD-15. Immunoreactivity of Epithelial Membrane Antigen (EMA) was noticed in the membranes of cells.
The data show that the cells obtained for cytological examination have the same characteristics as those in biopsy materials. Sarcomatoid mesothelioma may be limited of Cytokeratins. Reactions with Calretinin, Mesothelin and Thrombomodulin are the most important for Epithelial Mesothelioma with positive reactions with Vimentin, Keratins. Immunonegative reactions with CEA, Ber-EP4 and CD-15 are typical.

6540 POSTER

p53 gene mutation, mrna expression, aberrant protein expression and clinicopathological features in resected non-small cell lung cancer

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Background: Functional abnormality of p53 plays a pivotal role in occurrence of malignant tumors including lung cancer. Aberrant expression of the p53 protein using immunohistochemistry has been investigated in many cancers. However, immunohistochemical detection cannot distinguish expression of wild type p53 protein from mutant one, so that clinical significance of p53 aberrant expression should be analyzed with regard to the presence or absence of p53 gene mutations. In this study, we investigated relationships among gene mutation, mRNA expression and aberrant protein expression of the p53, and analyzed their clinical