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