better outcome was seen in pts without serum EGFR mutations. TTP was longer for pts with EGFR exon 19 deletions (not reached) than for pts with L858R (7.7 m) (P = 0.02). TTP for pts with PS 2 with exon 19 deletions was not reached, while it was 2.7 mo for pts with L858R (P = 0.17).
Conclusions: EGFR mutations in serum could be a non-invasive source of information on the genotype of the original tumor cells and could be a useful tool to predict patient response to erlotinib, especially in patients with poor PS.

6531 POSTER

XPD 312 single nucleotide polymorphism (SNP) predicts survival in stage IIIA−B non-small-cell lung cancer (NSCLC) patients (pts) <59 years (y) treated with chemotherapy followed by surgery

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Background: SNPs in DNA repair genes may affect response to cytotoxic therapy. We investigated SNPs in XPD codons 751 and 312 and in RRM1−37 in 109 stage IIIA (N2) and IIIB NSCLC pts treated with neoadjuvant chemotherapy and correlated results with event-free (EFS) and median (MS) survival.
Methods: Patients eligible for surgery received cisplatin day (d) 1, gemcitabine d 1, 8, docetaxel d 1, 8, 15, every 3 weeks for 3 cycles, followed by thoracotomy. DNA was extracted from baseline peripheral lymphocytes and genotyping was performed by Taqman.
Results: Median age, 60 y (range 31−77); 92 males (84%); 45 squamous cell (41%). 4 pts (3.9%) attained complete response; 55 (53.9%) partial response. 75 pts underwent surgery (62 complete, 13 incomplete resection); remaining 34 pts were unresectable. Median follow-up was 15.7 months (mo) (range, 0.5−74). MS for pts still alive is 49.8 mo (range, 6.7−74). MS: 48 mo with complete resection, 13 mo with incomplete resection, 17 mo for unresected pts. In the univariate analysis of survival, age <59 y (P = 0.03), resection (P < 0.001) and XPD312 AspAsp (P = 0.05) emerged as predictive markers of longer survival. For all 109 pts, those with XPD312 AspAsp had longer EFS and MS than pts with Asn variants (Table). In addition, for 51 pts <59 y, EFS was longer for 24 pts with XPD312 AspAsp (36.4 mo) than for 27 pts with Asn variants (9.8 mo) (P = 0.009); MS in this group of younger pts was 45.4 mo for AspAsp vs 15.8 mo for Asn (P = 0.04). No other significant correlation between SNPs and survival was observed (Table).
Conclusions: Interaction between SNPs, age and risk of lung cancer has previously been described. XPD312 AspAsp in pts <59 y predicts longer survival in stage IIIA (N2) and IIIB NSCLC treated with neoadjuvant chemotherapy.

	EFS			MS		
	N	m (95% CI)	p	N	m (95% CI)	p
XPD751						
LysLys	45	13.22 (3.49−22.95)	1.03	45	32.14 (5.08−59.20)	0.15
LysGln&GlnGln	64	8.82 (6.11−11.52)		64	14.90 (10.39−19.41)	
XPD312						
AspAsp	55	13.98 (4.79−23.17)	0.03	55	32.14 (7.58−56.70)	0.05
Asp&AsnAsn	54	7.34 (4.53−10.14)		44	12.04 (6.09−17.99)	
RRM1−37						
CC	59	9.11 (6.03−12.19)	0.87	59	14.97 (4.61−25.32)	0.53
CA&AA	49	10.79 (8.22−13.36)		49	16.84 (1.50−32.18)	

6532 POSTER

Zoledronic acid reduces invasion of different lung cancer cell lines

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Background: Zoledronic acid (ZOL) inhibits Ras farnesylation and thereby activation, however, an impact on invasion in lung cancer has never been studied. U-PAR, one of the most relevant metastasis-related molecules, is induced by Ras, among other stimuli. This study was performed to investigate an inhibition of u-PAR gene expression and invasion by ZOL in lung cancer cell lines.
Materials and Methods: Non Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC) cell lines were evaluated for their ZOL-IC50, and u-PAR expression was determined using qPCR (TaqMan). Inhibition of Ras activation was detected using Ras activation assays. Ras-codons 12, 13 and 61 (K-, H-, N-Ras) were amplified and sequenced. For u-PAR knockdown specific siRNA was used. Invasion was measured by matrigel assays.
Results: U-PAR mRNA did show either no change or even an increase after ZOL-treatment in NSCLC and SCLC. In contrast, we observed an expected 20% downregulation of u-PAR expression in the breast cancer cell line MDA-MB-231 (positive control). However, a second breast cancer cell line, MDA-MB-435, showed an 8-fold upregulation of u-PAR mRNA, while Ras activity was reduced in all cell lines. Ras sequence analysis did not reveal a correlation between the Ras-mutational status and the activating or inhibiting effect of ZOL on the expression of u PAR. Furthermore, specific siRNA-knockdown of u-PAR expression did not significantly affect ZOL-induced invasion. Nevertheless, matrigel invasion assays showed that the treatment with ZOL leads to a clear reduction of the invasive potential of lung cancer (35% reduction in H460, 53% in H1395, 60% in A549 at the IC50) and breast cancer cells (80% reduction in MDA-MB-231, 70% in MDA-MB-435).
Conclusions: These data suggest that 1. ZOL inhibits invasion in diverse lung- and breast cancer cell lines, 2. that this, however, is not primarily mediated via a suppression of u-PAR gene expression 3. that a potentially differential regulation of u-PAR via ZOL is not associated with Ras-mutations in codons 12, 13, and 61. Since the suppression of invasion by ZOL in NSCLC is independent of the u-PAR, the next step will be to screen for other invasion-related target genes of ZOL mediating its anti-invasive effect.

6533 POSTER

Cetuximab attenuates EGF induced u-PAR expression in NSCLC

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Background: Cetuximab is a chimeric IgG1 monoclonal antibody that blocks ligand binding to EGFR, leading to a decrease in receptor dimerization, autophosphorylation, and activation of signaling pathways. Here we investigate the potential of this drug to be used in NSCLC treatment and the possible mechanisms of the drug's activity on EGFR and thereby the invasion related molecule u-PAR.
Materials and Methods: MTT test was used to evaluate the effect of Cetuximab treatment on seven NSCLC cell lines. Wound healing assay was used to measure the effect of the drug on the cell motility. Cell cycle analysis was performed by FACS. Taqman qRT-PCR analysis was used to evaluate the expression of u-PAR mRNA. Luciferase reporter assay we used to evaluate u-PAR promoter activity. Transcription factors binding on u-PAR promoter was revealed by EMSA and supershift analysis.
Results: By using Cell proliferation (MTT) assay we characterized seven NSCLC cell lines for their permissiveness to Cetuximab treatment as sensitive (H1395, Calu3 and A427) and resistant (A549, LXF289, H1299 and H460). Further FACS analysis revealed that the reduced percentage in the cell growth in the sensitive cells (treated vs control) was due to the cell cycle arrest at G2/M phase of the drug treated samples. Cell

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