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