siRNA on human lung cancer cell lines, and the usage of its intravenous administration with atelocollagen as a drug delivery system (DDS) in a liver metastatic murine model.
Materials and Methods: Overexpression of PLK-1 in lung cancer tissues from patients was evaluated immunohistochemically and that in human lung cancer cell lines by western blotting. Growth inhibitory effects of PLK-1 siRNA were assessed by MTT assay, and cell death analysis by cytology, flow cytometry, and fluorometric caspase-3 analysis. We then transplanted luciferase labeled human non-small cell lung cancer cell line $A549^{LUC}$ to the spleens of BALB/c nu/nu mice so that these cells metastasized to livers via splenic vein. We treated this liver metastatic murine model with PLK-1 siRNA/atelocollagen complex for ten days from day 0 of transplantation. Tumor growth was evaluated by in vivo imaging system (IVIS) and macroscopically.
Results: PLK-1 overexpressed both in lung cancer tissues and in cell lines. Tissues from the patients with progressed stages and with poorly differentiated lung cancers expressed higher levels of PLK-1, and these patients presented with worse prognosis, suggesting PLK-1 expression reflects the prognosis. Growth inhibitory effects of PLK-1 siRNA were observed in a dose-dependent manner. SubG1 fractions, Annexin-V+/PI− and Annexin-V+/PI+ cells, and a caspase-3 activity increased after PLK-1 siRNA treatment, suggesting induction of apoptosis. Moreover, in vivo analysis showed PLK-1 siRNA/atelocollagen significantly inhibited the growth of liver metastatic tumors compared with PBS or nonsense siRNA/atelocollagen, which was confirmed by IVIS and also macroscopically.
Conclusions: PLK-1 siRNA showed growth inhibitory effects and apoptosis induction on lung cancer cells. Furthermore, PLK-1 siRNA/atelocollagen significantly inhibited the progression of liver metastases in murine model. These observations suggest that systemic siRNA/atelocollagen complex therapy can be an attractive and novel therapeutic strategy for liver metastasis in advanced lung cancer.

6528 POSTER

Role of ERCC1, XRCC3, Aurora A and TGFBR1 gene single nucleotide polymorphisms (SNP) and CHFR and 14-3-3σ methylation in a customized cisplatin (cis) trial based on ERCC1 mRNA levels in stage IV non-small-cell lung cancer (NSCLC) patients (pts)

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Background: The primary aim of this trial was response. In both the control arm and the genotypic arm with low tumor ERCC1 mRNA levels, pts received docetaxel(doc)/cis while in the genotypic arm with high tumor ERCC1 mRNA levels, pts received doc/gemcitabine. Response was significantly higher in the genotypic arms. We examined 324 pts for genetic markers that could influence response, including ERCC1 118 C/T, ERCC1 C8092A, XRCC3 241 (Thr to Met), Aurora A 91 T>A, Aurora A 169G>A, a SNP within intron 7 of the TGFBR1 gene (Int7G24A), and an in-frame germline deletion (TGFBR1*6A). Methylation of 14-3-3σ and CHFR were also analyzed.
Methods: DNA from peripheral lymphocytes was used for genotyping (Taqman assay) and methylation-specific PCR was used for 14-3-3σ and CHFR in pretreatment serum DNA.
Results: There were no differences between clinical characteristics and the different SNP types, except that Aurora A 91 AA type had higher tumor ERCC1 mRNA levels (P = 0.005). No relationship was found between ERCC1 SNPs and tumor ERCC1 mRNA levels. A strong correlation was found between the Int7G24A and XRCC3 241 SNPs (P = 0.03). The Int7G24A GA type had a higher odds ratio (OR) of response (OR 2.32, P = 0.02); the OR for the AA type was 3.15. XRCC3 241 MetMet had lower probability of response (OR 0.23, P = 0.04). Neither other SNPs nor methylation influenced response. The best multivariate model for response was observed in pts with PS 0, low ERCC1 levels, and XRCC3 241 SNP (Table).
Conclusions: Further research is warranted to define the role of theTGFBR1 Int7G24A gene in customized treatments.

6529 POSTER

14-3-3σ and checkpoint with forkhead and ring finger (CHFR) methylation in serum in erlotinib-treated non-small-cell lung cancer (NSCLC) patients (pts) with EGFR mutations

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Background: 14-3-3 proteins have 130 potential binding partners, including Cbl. 14-3-3 expression can prevent mutant EGFR binding to Cbl, impairing ubiquitination and endocytosis. 14-3-3σ is frequently methylated in NSCLC; we hypothesized that in the presence of EGFR mutations, methylated 14-3-3σ could permit the formation of the EGFR-Cbl complex. CHFR is a checkpoint that delays entry into metaphase in response to mitotic stress.
Methods: 73 stage IV NSCLC pts with EGFR exon 19 deletion or exon 21 L858R mutation received first- or second-line erlotinib single therapy. 14-3-3σ and CHFR methylation was examined in the baseline serum of these pts.
Results: Median age, 63 (range, 26−83); females, 48 p (65.8%); Caucasian, 72 p, Asian, 1 pt; never-smokers, 45 pts, ex-smokers, 21 pts, smokers, 7 pts; adenocarcinoma, 64 pts, large cell carcinoma, 9. PS: 0, 19 pts, 1, 42 pts, 2−3, 12 pts. 14-3-3σ was methylated in 39.7% and CHFR in 42.5% of pts. No differences in patient characteristics were observed according to methylation status. Complete response was observed in 11.1% of pts, and partial response in 75.4%. Overall response was 86.5%. There was a trend toward a higher response rate in pts with unmethylated CHFR (94.4% vs 76.6%; P = ns). Overall median time to progression (TTP) and survival (MS) have not been reached either in first- or second-line. However, when split according to methylation status, there was a trend toward better TTP and MS in both first- and second-line in pts with methylated 14-3-3σ. TTP in second-line in pts with methylated 14-3-3σ has not been reached, while it was 10.8 months (mo) for pts with unmethylated 14-3-3σ (P = ns). TTP in second-line in pts with methylated CHFR was 5.2 mo but was not reached for pts with unmethylated CHFR (P = 0.05).
Conclusions: Methylated 14-3-3σ can permit Cbl binding to mutant EGFR and predict longer-lasting response to erlotinib in pts with EGFR mutations. The precise role of CHFR warrants further research. Complete data will be presented.

6530 POSTER

High correspondence between EGFR mutations in tissue and in circulating DNA form non-small-cell lung cancer (NSCLC) patients (pts) with poor performance status (PS)

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Background: We evaluated the correspondence between EGFR mutations in NSCLC tissue and matched serum DNA and the value of EGFR mutations as a serological marker.
Methods: 121 Spanish stage IV NSCLC pts received customized first- or second-line erlotinib monotherapy based on the presence of EGFR mutations in the tumor tissue. Serum genomic DNA was obtained from all pts prior to erlotinib administration. EGFR exon 19 deletions were studied by length analysis of fluorescently labeled PCR products and the exon 21 L858R mutation by a PCR Taqman assay.
Results: The EGFR mutation status in the serum was consistent with that in the tumor tissue of 82/121 pts (68%) and of 15/16 pts (93.8%) with PS 2 had mutations. Overall, 64.3% of pts had an exon 19 deletion and 35.7% had L858R. 78% of mutations were found in females (P = 0.01) and 77.6% in never-smokers (P = 0.07). Response rate was 88% in pts with mutations only in the tumor and 87% in pts with mutations in tumor and serum. Complete responses were observed in 20% of pts with mutations in tumor and serum vs 4% of pts with mutations only in tumor (P = 0.09). With a median follow-up of 6.8 months (mo) (range, 1.2−17.6), time to progression (TTP) and median survival have not been reached. A trend to

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