50 μM LY in both cell lines. Both cell lines expressed Bcl-X (L) and Bcl-2, but the effect of LY was ambiguous.
Conclusions: Effective inhibition of P-Akt corresponded to an increased apoptosis in wt cells, but not to caspase activation. In res1.2 cells, efficient inhibition of P-Akt never occurred, and there was no change in apoptosis. The inefficient inhibition of P-Akt by LY indicated that PI3-kinase is either strongly overactivated or that PI3-kinase is not the main phosphorylator of Akt 1 in P31 cells. The role of the anti-apoptotic proteins Bcl-X (L) and Bcl-2 in survival of P31 cells needs to be further investigated. To conclude, P31 res1.2 cells appeared to be more resistant to LY inhibition of P-Akt than P31 wt cells. In P31 wt cells, P-Akt was important for survival and affected caspase-3 activity. The involvement of PI3-kinase and Akt/PKB in the cisplatin-induced apoptosis signalling pathways of P31 wt and res1.2 cells remain to be elucidated.

1155 POSTER

EGFR mutations in NSCLC: Genotypic analysis and implementation of complementary screening tests for detection purposes

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Background: Somatic mutations of the epidermal growth factor receptor (*EGFR*) gene in non-small cell lung cancer (NSCLC) predict responsiveness to the EGFR tyrosine kinase inhibitors. These mutations are commonly identified using DNA sequencing methods. Although considered the gold standard, this approach requires a high ratio of tumor to normal tissue DNA for optimal results which is not often available in biopsies obtained from these patients. Due to this limitation, we have applied selected screening tests to enhance the sensitivity of DNA sequencing.
Materials and methods: Clinical specimens from 50 NSCLC patients were analysed for *EGFR* mutations in exons 18, 19, and 21. After DNA extraction and PCR, mutations were examined by sequencing genomic DNA. Additionally, PCR products were screened for exon 19 deletions using a fragment analysis strategy.
Results: Sequencing revealed 5 mutations: 3 missense mutations in exon 21 and 2 deletion mutations in exon 19. Fragment analysis of the samples detected the original 2 deletion mutations and an additional 4 new exon 19 deletion mutations that were further confirmed by direct sequencing with re-designed PCR primers. In our hands, fragment analysis was able to detect mutations in samples containing as little as 10% mutated DNA whereas direct sequencing requires at least 30%.
Conclusion: Clinically relevant mutations in the *EGFR* gene may not be detected using sequencing techniques because of insufficient tumor DNA in biopsy samples. The application of additional rapid and more sensitive screening tests may be able to overcome this limitation. Fragment analysis is a quick and reliable method for the detection of *EGFR* exon 19 deletion mutations in lung cancer that may be missed by standard DNA sequencing methods. Fragment analysis to detect deletion mutations and other more sensitive screening tests to detect missense mutations should be implemented as complimentary methods for detection of *EGFR* mutations.

1156 POSTER

Identification of prognostically significant subsets of stage IIIA N2 non-small cell lung cancer patients by hierarchical clustering analysis of tissue microarray immunostaining. An Alpe-Adria Thoracic Oncology Multidisciplinary group study (ATOM 014)

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Background: Based on gene expression profiling, prognostically relevant subsets of patients have been identified for breast cancer, lung cancer and lymphoma. In the future, gene expression profile of each individual patient might provide support for tailored therapeutic decision making.
Methods: We performed a hierarchical clustering analysis of tissue microarray (TMA) immunostaining data of 87 patients with stage IIIA pN2 non small cell lung cancer (NSCLC), treated with radical surgery between 1985 and 1997. The expression of the following markers was evaluated: EGFR, ErbB-2, c-kit, COX-2, survivin, bcl-2, cyclin D1, cyclin B1, MMP-2, MMP-9 and univariate, multivariate analyses and unsupervised hierarchical clustering analysis by using these 10 markers were performed.
Results: Bcl-2 ($p < 0.0001$) and cyclin D1 ($p = 0.0036$) are more expressed in squamous cell carcinoma (SCC), while MMP-2 ($p = 0.0115$), MMP-9 ($p = 0.0075$) and survivn ($p = 0.02$) display increased expression levels in histological subtypes other than SCC. In univariate analysis, only squamous cell histology, bcl-2 and cyclin D1 expression were favorable prognostic factors ($p = 0.0149$, $p = 0.0013$, $p < 0.0001$, respectively), while MMP-2 expression was associated with worse prognosis ($p = 0.013$). In multivariate analysis, cyclin D1 and MMP-2 were the only positive and negative prognostic factors, respectively ($p < 0.0001$, $p = 0.06$). Unsupervised hierarchical clustering analysis of TMA immunostaining data produced 5 distinct cluster groups and the deduced tree identified 2 prognostically significant subsets of patients, with better (groups 1–2) and worse (groups 3–4–5) prognosis in terms of median survival (51 vs. 10 months, $p < 0.0001$). Notably, groups 1–2 were mostly composed of SCC (80%).
Conclusions: These results suggest that hierarchical clustering of TMA immunostaining data by using a limited set of markers might provide a useful tool for the identification of radically resected NSCLC patients at high risk of recurrence, likely to benefit from more aggressive treatment.

1157 POSTER

Expression of hypoxia-inducible factor-1 alpha and its prognostic significance in small-sized adenocarcinomas of the lung

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Objective: To analyze the prognostic value of hypoxia-inducible factor-1 alpha expression and its correlation with clinicopathologic variables and expression of vascular endothelial growth factor-A, -C, and R-2 in patients with lung adenocarcinomas of small size.
Methods: The expression of hypoxia-inducible factor-1 alpha was immunohistochemically determined in 78 cases of small-sized adenocarcinoma (maximum dimension is less than 2 cm) using polyclonal antibody against a recombinant protein corresponding to amino acids 575–780 of hypoxia-inducible factor-1 alpha. Data regarding patient survival, clinicopathologic factors, and immunohistochemical studies of vascular endothelial growth factor were also collected.
Results: Strong expression of hypoxia-inducible factor-1 alpha was observed in 29 (37%) of 78 cases; no expression was found in the bronchioalveolar carcinomas. Strong expression of hypoxia-inducible factor-1 alpha was significantly higher in cases with vascular invasion, lymphatic permeation, lymph node involvement, and advanced pathological stage. Strong expression of hypoxia-inducible factor-1 alpha was correlated with strong expression of vascular endothelial growth factor-A, -C, and R-2. The 5-year survival rate was 69% if expression of hypoxia-inducible factor-1 alpha was strong and 84% if expression was weak. Multivariate analysis revealed that pathological N status and pleural invasion were independent prognostic factors and strong expression of hypoxia-inducible factor-1 alpha was marginal significance.
Conclusions: Strong expression of hypoxia-inducible factor-1 alpha was associated with vascular invasion, lymphatic permeation, nodal involvement, pathological stage, and strong expression of vascular endothelial growth factor-A, -C, and R-2. Strong expression of hypoxia-inducible factor-1 alpha was a poor prognostic factor for patients with small-sized adenocarcinoma of the lung.

1158 POSTER

Can 18FDG-PET/CT scan be used to define a biological target volume (BTV) for IMRT treatment planning of non-small cell lung cancer patients?

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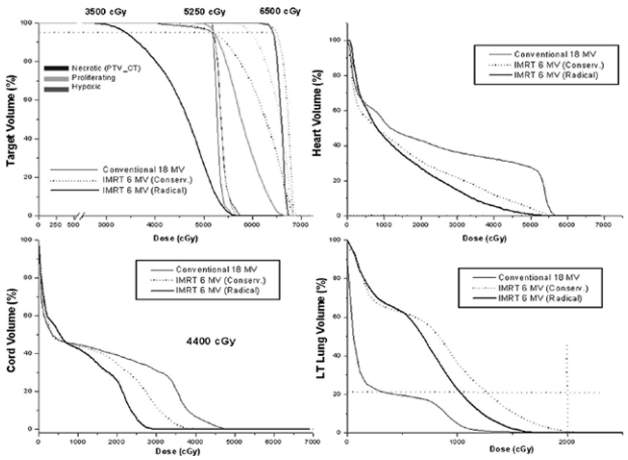
Purpose: To test the feasibility of FDG based PET/CT data on target volume delineation in radiotherapy treatment planning of NSCLC patients, and impact of these outlined biological target volumes (BTV) for IMRT treatment.
Materials and methods: Patient diagnosed with non-operable NSCLC in the right upper lobe had a 3D conformal planning based on CT data with our hypo-fractionated regimen of 52.5 Gy in 15 fractions. Planning was

redone with fusion of PET/CT data and 3D CT. Three target volumes were created: necrotic BTV (same as seen in CT), proliferating BTV (based on PET signal to background ratio 1:3) and hypoxic BTV (based on PET signal to background ratio of 1:19, believed to be related to anaerobic glycolisis inefficacy in ATP production). Two IMRT plans were created based on these three BTVs. The first plan ("conservative plan") delivers 52.5 Gy to the necrotic BTV and 65 Gy to the hypoxic BTV. The second plan ("radical change") delivers 30 Gy to the necrotic BTV, 52.5 Gy to proliferating BTV and 65 Gy to hypoxic BTV.
Results: Impact of different target volumes on DVH curves for the three BTVs and the critical structures are shown in Figure 1. The use of BTVs in IMRT plan seems attractive because it increases dose to targets considered to need higher doses. It reduces considerably dose to the heart and spinal cord, organs considered to limit dose escalation approaches in NSCLC treatment. However, lower dose to the spinal cord comes at the expense of slight increase in the contra lateral lung dose, still way below V20 limit.



Conclusions: The "conservative" IMRT approach can be understood as a PET/CT based concomitant boost to the tumor expressing the highest FDG uptake. The "radical" IMRT delivery implies a deviation from the traditional uniform dose target coverage approach, with the intention of achieving a better surrounding tissue sparing and ultimately allowing for dose escalation protocols in NSCLC patients. Several issues should be considered before treating patients using PET/CT based BTVs: tumor motion (4D PET/CT scanning and gated RT), dose calculation accuracy with Monte Carlo based treatment planning, and specific tumor metabolic activity imaged with better radiopharmaceutical markers. We also intend to present four current recommendations for tumor outlining using PET: Qualitative Visual Method (Ciernik), CTV = 2.5 SUV units (Paulino and Johnstone), CTV = 40% Iso of max Uptake Value (Erdi) and Linear SUV threshold function method (Black).

1159 POSTER

Insulin-like growth factor receptor 1 (IGFR-1) expression is significantly associated with longer survival in non-small cell lung cancer (NSCLC) patients treated with gefitinib

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Background: Clinical data have already demonstrated that the prevention of Epidermal Growth factor Receptor (EGFR) mediated signal transduction by the small molecule inhibitor gefitinib provides a promising new treatment option for patients with NSCLC, especially for those with specific EGFR gene mutations or amplification. IGFR-1 is a transmembrane Tyrosine Kinase (TK) receptor implicated in promoting oncogenic transformation, growth, and survival of cancer cells. Data on cell lines suggested that IGRF-1 mediate resistance to anti-EGFR therapy through continued activation of the antiapoptotic PI3K-akt pathway. In NSCLC no data exists on IGFR-1 expression and on its effects on gefitinib therapy.
Materials and Methods: A total of 77 NSCLC patients treated with gefitinib were evaluated for IGFR-1 expression by immunohistochemistry in tumor samples collected at the time of original diagnosis. Using a semiquantitative scoring system, patients were classified as IGFR-1− (score <100) or IGFR-1+ (score 100 to 400). This cohort included 59 patients previously evaluated for EGFR, HER2, HER3, and Phospho-Akt.
Results: IGFR-1 resulted positive in 30 and negative in 47 cases. IGFR-1 expression was not significantly associated with any clinical characteristic, such as gender, histology, or smoking history. No association was also found with other biological markers, such as EGFR gene mutation, amplification or expression, HER2 amplification, HER3 amplification and Phospho-Akt expression. No difference in response (16.7% versus 12.8%, $p = 0.74$) and time to progression (2.6 versus 3.06 months, $p = 0.83$) were observed in IGFR-1+ and IGFR-1−. Median survival was significantly longer in IGFR-1+ patients (17.8 versus 7.3 months, $p = 0.013$). Multivariable analysis confirmed that IGFR-1 negative status was significantly associated with higher risk of death (Hazard Ratio 2.21, $p = 0.012$).
Conclusions: In NSCLC patients treated with gefitinib, IGFR-1 expression is not associated with gefitinib sensitivity in terms of response and time to progression but significantly associated with longer survival. The role of IGFR-1 as independent prognostic factor should be validated in a cohort of NSCLC not treated with TK inhibitors.

1160 POSTER

CXCL12−3'A polymorphism and Lung Cancer metastases protection: new perspectives in immunotherapy?

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Background: The Stromal Derived Factor-1 (SDF-1/CXCL12) chemokine and its receptor CXCR4 have been implied in the development of long distance metastases of several types of cancers, including Non-Small Cell Lung Cancer (NSCLC). A single nucleotide polymorphism consisting in a G to A transition in the UTR3' of *CXCL12* gene, *CXCL12−3'A* polymorphism, and its allelic frequencies were associated with breast cancer and melanoma. The objective of this study was the evaluation of the genetic influence of the *CXCL12−3'A* polymorphism in the susceptibility to lung cancer development.
Material and methods: DNA samples were extracted from peripheral blood cells of 403 patients (154 patients diagnosed with lung cancer and 249 healthy individuals). The *CXCL12−3'A* polymorphism was analyzed through PCR-RFLP (*MspI*). 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 *CXCL12−3'A* genotypes and lung cancer risk.
Results: Our data showed that the presence of A carrier genotypes was more frequent in patients with epidermoid NSCLC without long distance metastases, (46.6%), than in patients with epidermoid NSCLC with long distant metastases, (20%). This difference is statistically significant ($P = 0.036$) and suggests that patients with epidermoid NSCLC carrying the A allele present almost 3.5 times less risk of developing long distance metastases (OR = 0.29; 95%CI = 0.09−0.97). Multivariate logistic regression analysis indicates that the A allele presence (aOR = 0.221; 95%IC 0.056−0.877; $P = 0.032$) and age at diagnosis above 66 years (aOR = 0.220; 95%IC 0.067−0.728; $P = 0.013$) are significantly associated with the development of epidermoid NSCLC with long distance metastases.
Conclusions: Our findings suggest that *CXCL12−3'A* polymorphism has a protective role in the development of NSCLC metastases and the elucidation of the molecular mechanisms underlying this protective effect could open the possibility of creating an effective immunotherapeutical approach that mimicries the effects of the polymorphism.

1161 POSTER

VEGF and S100 beta serum levels in advanced non-small cell lung cancer (NSCLC) patients with and withour brain metastases (BM) at diagnosis

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Background: BM at diagnosis is found in 25−30% of NSCLC patients (pts) and are frequently associated with abnormal blood-brain barrier (BBB) function. Serum levels of calcium-binding, astro-glial protein S-100 beta are suggested to be an important marker of BBB-integrity. Data have also shown the role of VEGF in BBB-integrity and in BM growth. The study is aimed to explore if there is any difference between serum levels of S100B and VEGF in advanced NSCLC pts with and without BM at diagnosis.
Patients and Methods: Peripheral blood samples from 73 metastatic NSCLC pts (37 without and 36 with BM) were collected. Pts in both