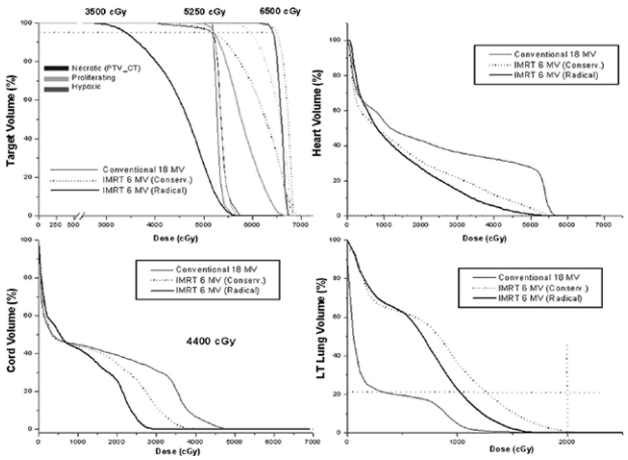
redone with fusion of PET/CT data and 3D CT. Three target volumes were created: necrotic BTV (same as seen in CT), proliferating BTV (based on PET signal to background ratio 1:3) and hypoxic BTV (based on PET signal to background ratio of 1:19, believed to be related to anaerobic glycolisis inefficacy 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

groups were equally distributed according to age, sex, histology, ECOG PS and co-morbidity (hypertension, diabetes and VTE). VEGF serum levels were detected by a commercially available ELISA kit (Quantikine Human VEGF Immunoassay R&D systems, Minneapolis, MN, USA) according to the manifacturer's instructions. S100 B serum levels were measured by the enzyme immunoassay Kit NEXUS Dx ™ (SynX Pharma Inc., Toronto, Canada).

Results: Median age was 60.5 years (range 24–79), M/F 57/16, squamous/adeno/large-cell/non specified NSCLC 18/19/11/26, ECOG PS ⩽2: 64 pts (33 pts with BM and 31 pts without BM), ECOG PS 3: 9 pts (5 pts with BM and 4 pts without BM). Three pts had only BM, while the other were plurimetastatic: bone (24 pts (lung (21 pts), liver (11pts) and adrenal glands (16 pts). The mean value (±SD) of serum VEGF was 506 (±432) pg/ml. VEGF levels showed significant association with large cell histotype ($p = 0.041$). Baseline VEGF serum levels were also correlated with ECOG PS ($p = 0.040$) (pts with ECOG PS 0 had lower VEGF levels (417±364 pg/ml) while pts with ECOG PS 3 had higher VEGF levels (944±699 pg/ml). There was also an association between white blood cells and ECOG PS status ($p = 0.040$). An inverse correlation between serum VEGF levels and haemoglobin was found ($r = -0.24$, $p = 0.046$). No difference in VEGF serum levels was found between pts with BM [501 (±443) pg/ml] and without BM [511(±427) pg/ml] ($p = 0.9$). There was a difference between mean VEGF values in the subgroup of pts with only one BM (300±231 pg/ml) vs pts with multiple BM (506±332 pg/ml) even if it doesn't reach a statistical significance ($p = 0.069$). The S100B serum levels for both groups were <0.01 ng/ml.

Conclusions: No significant association was found between S100 beta levels and the patients' clinical parameters. In particular S100B levels does not seem to discriminate pts with and without BM. Elevated baseline serum VEGF levels appear to be related with the degree of metastatic involvement.

1162 POSTER

Weekly Combretastatin A4 Phosphate (CA4P) in combination with radiotherapy (RT): tumour antivascular effects as demonstrated using perfusion computed tomography (p-CT)

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Purpose: The vascular disrupting agent CA4P, when used as a single agent, causes transient reduction in tumour perfusion. CA4P may act synergistically with RT. Tumour vascular changes that occur during treatment with once-weekly CA4P in combination with RT have been measured using p-CT.

Methods/Materials: Following Local Research Ethics Committee approval and written informed consent, patients with histologically confirmed, advanced non small cell lung cancer were enrolled into a Phase IB clinical trial of CA4P combined with external beam RT. They received twice-weekly palliative RT (27 Gy in 6 fractions) over three weeks. Six patients in the first cohort received a single dose of CA4P (50 mg/m^2) after the first 2nd fraction of RT. Six patients in the second cohort received the same dose of CA4P after the 2nd, 4th, and 6th fractions of RT. Quantitative p-CT measurements of whole tumour blood volume and permeability were performed prior to treatment, after RT, before CA4P and at 4 and 72 hours after every CA4P dose.

Results: After the 2nd, 4th and 6th fraction of RT prior to CA4P, tumour blood volume increased by 13% (paired t-test, $p = 0.16$), 34% ($p = 0.06$), and 26% ($p = 0.05$) respectively. Increases in permeability were seen after RT but failed to reach significance. 4 and 72 hours after receiving the 1st dose of CA4P, tumour blood volume decreased by 15% ($p = 0.03$) and 19% ($p = 0.02$) respectively; after the 2nd dose by 9% ($p = 0.04$) and 32% ($p = 0.02$) respectively; and after the 3rd dose by 7% ($p = 0.3$) and 23% ($p = 0.06$) respectively. At the end of treatment, there was an overall reduction in blood volume of 33% from baseline ($p = 0.04$). Increase in permeability after RT correlated to subsequent reduction in blood volume after CA4P ($r = 0.76$, $p = 0.004$).

Conclusion: Weekly CA4P with RT caused a sustained reduction in tumour blood volume that is measurable using p-CT. Repeated doses of CA4P resulted in additional decrease in blood volume. Changes in tumour permeability after RT may predict for subsequent tumour response to CA4P. There is potential synergy between CA4P and RT.

1163 POSTER

Relation between P53 codon 72 polymorphism and somatic P53 gene mutation in non-small cell lung cancer (NSCLC)

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Background: *P*53 gene mutation is among the most frequent molecular abnormalities in lung cancer and is strongly associated with cigarette smoking. A relation between *P*53 gene polymorphism and mutation was postulated recently in breast carcinoma but data on this subject in NSCLC have been scarce. The aim of this study was to assess the association between constitutional pro72codon polymorphic variant of *P*53 gene and the risk of somatic *P*53 gene mutations in NSCLC.

Material and methods: Study group included 240 NSCLC patients (52 females and 188 males) who underwent curative pulmonary resection between 1996 and 2000. Arg72Pro *P*53 polymorphism analysis was performed using peripheral blood samples. In 31 NSCLC cases for whom blood samples were not available, tumor-free lung tissue was used for polymorphism analysis. *P*53 gene codon 72 polymorphism was evaluated by allele specific amplification-polymerase chain reaction (ASA-PCR) with Taq DNA polymerase and allele-specific primers. The results were confirmed by denaturing high-performance liquid chromatography (DHPLC). Somatic *P*53 mutation analysis included sequencing of exons 5–8 in tumor DNA.

Results: The frequencies of *P*53 gene Arg72Pro genotypes (Arg/Arg, Arg/Pro, Pro/Pro) in NSCLC patients were 46%, 50% and 4%, respectively. No relationship was found in NSCLC patients between polymorphic variants and clinical characteristics, such as age, sex, pT, pN, histological type and smoking, and neither was there a correlation between polymorphic variants and overall survival. *P*53 gene somatic mutations were found in 76 out of 240 NSCLC patients (32%). Most common were missense mutations. There was no correlation between *P*53 somatic mutations and overall survival. The mutations were more frequent in pro72codon carriers (49/130 patients – 38%) than in arg72codon homozygotes (27/110 patients – 25%). The odds ratio for mutations of *P*53 gene in tumor cells in Pro allele carriers was 1.80 (95% CI: 1.03–3.16).

Conclusions: Arg72Pro *P*53 gene polymorphism may increase the risk of somatic *P53* gene mutations in NSCLC patients. The biological significance of this finding warrants further studies.

1164 POSTER

Can brain metastases (BM) timing presentation influence on survival in patients with lung cancer treated by radiosurgery (RS) ? Long term results

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Purpose: To determine if BM timing presentation (synchronous vs. metachronous) in patients affected of lung cancer treated by RS can influence on overall survival (OS).

Patients and Methods: One hundred consecutive patients (p) have been included; 10% of cases had SCLC. Synchronous BM were observed in 43 cases, and metachronous in 57cases. KPS were as follows: 10 p had 60, 23 p had 70, 34 p had 80, 23 p had 90, and 10 p had 100. Only in 6 p primary tumour was considered in progression. RTOG RPA classes were: class I 31 p, class II 59 p, and class III 10 p. Ninety one percent of patients received WBI (sequential in 63 p). Number of BM previous RS were: one in 59 cases, two in 29 cases, three in 8 cases, and four in 4 cases. Median treated BM volume for RS was 2.7 cc (0.1–23.8). RS doses administered at isocenter was: 22.6 Gy (19–27.3).

Results: median follow-up was 19 m (2–124) (6% lost of follow-up); 28 p were alive at the end of analysis. KPS at last F-U was: 40–60 in 13 p, 70–100 in 14 p, and unknown in 1 p. Median survival time since RS was 10.3 months.

Local control at final analysis: 40 cases (11% in CR) did not progress in brain, 46 progressed, and 14 can not be evaluated because of rapid lethal events. Six patients received RS again as a salvage treatment.

Causes of death: 25 p because brain progression, 26 p because systemic progression, 17 p both causes, 1 p due to intercurrent disease, and 3 p with unknown reason.

On multivariate analysis we observed, as independent prognostic variables for OS, progression of the primary tumour (RR 4.38, CI 1.7–10.9), KPS 60–70 (RR 2.32, CI 1.4–3.9), and absence of WBI (RR 2.8, CI 1.1–6.9).

When we performed an exclusive analysis for NSCLC patients, MST was 10.6 months, and multivariate analyses showed that progression of the primary tumor (RR 4.87, CI 1.7–13.5), and KPS 60–70 (RR 2.16, CI