significance as well as prognostic impact in resected non-small cell lung cancer (NSCLC) patients.
Patients and Methods: A total of 112 patients with p-stage I-IIIB NSCLC without any preoperative therapy were included in this study. 76 patients (67.9%) received postoperative adjuvant chemotherapy, 64 with oral administration, 4 with systemic chemotherapy, and 8 with both. p53 gene mutations within exon 5, 6, 7 and 8 were screened using PCR single-strand conformational polymorphism method, and were determined with direct sequencing. The expression level of p53 mRNA was measured using quantitative real-time RT-PCR. Aberrant expression of p53 protein was evaluated with immunohistochemical staining. The clinicopathological parameters and p53 status were integrated to statistical analyses including overall survival and disease free interval.
Results: p53 gene mutation was observed in 33 cases (29.5%) including 3 cases with multiple mutations. Aberrant expression of p53 protein was demonstrated in 50 cases (47.1%). p53 mRNA expression was higher in cases with p53 aberrant expression than in cases without aberrant expression ($p = 0.005$). In wild-type p53 adenocarcinoma cases, mRNA expression decreased in order of differentiation status (well > moderate > poor), and was higher in node negative cases than in node positive cases ($p = 0.036$), although that of mutant p53 adenocarcinoma or other histological types did not show such tendency. There was no prognostic impact in any of single parameter such as gene mutation, mRNA expression and aberrant protein expression in multivariate analysis.
Conclusions: The wild-type p53 mRNA expression level is associated with tumor differentiation and nodal status in lung adenocarcinoma patients.

6541 POSTER

Genetic polymorphism of the epidermal growth factor gene – value for the treatment of non-small cell lung cancer (NSCLC)

A. Araújo[1], A.P. Araújo[2], A.L. Teixeira[2], A. Coelho[2], R. Ribeiro[2], I. Azevedo[1], R. Catarino[2], D. Pinto[2], M. Soares[1], R. Medeiros[2]. *[1]Instituto Português de Oncologia-Porto, Medical Oncology, Porto, Portugal; [2]Instituto Português de Oncologia-Porto, Molecular Oncology – CI, Porto, Portugal*

Background: In western world, lung cancer is the third type of cancer and non-small-cell lung cancer (NSCLC) accounts for 80% of all lung cancers representing the leading cause of death from cancer. The epidermal growth factor (EGF) has an established important role in lung carcinogenesis. EGF+61G/A is a biallelic G/A functional polymorphism, located in the 5′-UTR, which leads to increased EGF expression. The aim of our study was to evaluate the genetic influence of this polymorphism in NSCLC development.
Material and Methods: DNA samples extracted from peripheral blood cells of 171 patients (pts) with NSCLC, with an accurate stage and a 3 month minimum of follow-up, were analyzed. From 171 pts, with a mean age 62.7 years (median 64.0), 136 were males, 131 had a smoking history, and 85 had adenocarcinoma. The EGF genotypes were determined using the PCR-RFLP methodology.
Results: Regarding the frequency of the EGF+61G/A polymorphism genotypes, 63.2% of patients showed genotypes carrying the G allele and 36.8% presented the homozygous genotype AA. Among G carrier genotypes, 16.7% corresponded to NSCLC patients with stages I/II and 83.3% to advanced stages of the disease (III/IV). Regarding AA genotype, 30.2% of the patients were diagnosed with early stage NSCLC (I/II) and 69.8% presented advanced stages of NSCLC (III/IV). These differences were statistically significant and suggest that individuals with genotypes carrying the G allele present a 2.16-fold higher risk for the progression from early stages of NSCLC (I/II) to clinically more advanced stages of the disease (III/IV) (OR = 2.16; 95% CI: 1.03−4.52; $P = 0.039$).
Conclusions: These preliminary results indicate that the EGF+61G/A is involved in NSCLC progression, which is in agreement with previous findings that suggest that EGF overexpression is associated with worst prognosis of the disease. This makes EGF polymorphism an attractive factor for prognosis in NSCLC.

6542 POSTER

FISH and immunohistochemical analysis of PTEN in human mesothelioma cell lines

P.G. Betta[1], R. Libener[1], M. Salvio[1], G. Bottero[2], B. Castagneto[3], E. Piccolini[4], S. Orecchia[1]. *[1]Pathology Unit, ASO Alessandria, Alessandria, Italy; [2]Medical Oncology Unit, ASO Alessandria, Alessandria, Italy; [3]Medical Oncology Unit, ASL 22, Novi Ligure, Italy; [4]Pneumology Unit, ASL 21, Casale M.to, Italy*

Background: Pleural Malignant Mesothelioma (MM) is a highly aggressive and rapidly fatal tumour that is resistant to conventional chemotherapy. New molecular signalling pathways in MM are being explored, aimed at new, more effective treatment strategies. PTEN (phosphatase and tensin analog), a tumour suppressor, has been implicated in a large number of human tumours. PTEN is a phosphatase that can modulate signal-transduction pathways. At least part of its role is to regulate the activity of the serine/threonine kinase AKT and thus influence cell survival signalling. A recent study has shown elevated AKT activity in 65% of human MM specimens and in a human MM cell line exhibiting loss of PTEN. In the present in-vitro study, a possible role of PTEN in MM tumourigenesis is investigated.
Materials and Methods: PTEN protein expression was investigated by an immunocytochemical analysis using a commercial MAb (clone 28H6; Lab Vision Co.) in 12 human MM cell lines established from pleural effusions of histologically confirmed MM patients. Dual colour FISH using DNA probes for cytoband 10q23.3 (PTEN locus) and region 10p11.1-q11.1 (centromere of chromosome 10) (LSI PTEN/CEP10; Vysis Inc.) was performed to assess the PTEN gene status.
Results: A predominantly nuclear PTEN staining was observed in 7 of 12 (58.3%) MM cell lines. In the other 5 MM cell lines no PTEN expression was detected. Of these 5 PTEN negative cell lines, 2 showed the loss of a PTEN gene allele.
Conclusions: These data show that the loss of functional PTEN occurs in 41.7% of MMs and the down-regulation of PTEN protein can be related in a minority of cases (2 of 5) to loss of heterozigosity (LOH). One copy of PTEN may be haploinsufficient and the 50% reduction of gene function due to loss of one allele results in an abnormal phenotype. Since LOH can rarely be detected in MM, different mechanisms may be responsible for PTEN protein deregulation, such as inactivating mutations, protein instability, promoter hypermethylation and unknown epigenetic mechanisms. These findings are an important consideration for novel therapeutic trials in MM in which biological efficacy is influenced by the activity level of PTEN.

6543 POSTER

Molecular markers expression in mediastinal nodes from resected stage I non-small cell lung cancer (NSCLC): prognostic impact and potential role as markers of occult micrometastasis

S. Benlloch[1], J. Galbis[2], C. Alenda[3], F.M. Peiro[3], J.L. Marti[4], B. Baschwitz[5], J.M. Rodriguez-Paniagua[5], R. Rosell[6], M. Taron[6], B. Massuti[4]. *[1]Hospital General de Alicante, Research Unit, Alicante, Spain; [2]Hospital de la Ribera, Thoracic surgery, Alzira, Spain; [3]Hospital General de Alicante, Pathology, Alicante, Spain; [4]Hospital General de Alicante, Medical Oncology, Alicante, Spain; [5]Hospital General de Alicante, Thoracic surgery, Alicante, Spain; [6]Hospital Germans Trias i Pujol Institut Català d'Oncologia, Medical Oncology, Alicante, Spain*

Background: 5-year survival in surgically resected Stage I NSCLC is 60−70% whereas in cases with affected lymph nodes (LN) is <50%. The main risk factor for recurrence is nodal disease. Current histopathological analysis can miss occult micrometastases in nodal tissues at initial diagnosis. Detection of micrometastases with a more sensitive technique would be useful to define a high-risk population selecting patients (p) for postoperatory treatment. We assessed the role of several genes mRNA expression in pathological negative LN from resected Stage I NSCLC p as markers of occult micrometastases and correlated the results with relapse, and survival
Materials and Methods: Paired tumor and histological negative LN ($n = 344$) obtained by systematic mediastinal lymphadenectomy from 38 surgically resected Stage I NSCLC p were analyzed for the presence of 12 genes mRNA expression using RT-Q-PCR in an ABIPRISM 7500. RNA was extracted using ABIPRISM 6100. Specifically designed primers and probes were purchased from Applied Biosystems as Assay-on-demand; GADPH was used as an endogenous control. Samples were also analyzed by ICH for LN staging.
Results: 38 NSCLC p; 12 adenocarcinoma, 16 squamous cell, 10 undifferentiated. From the 12 tested genes CEA and PLUNC were found with high expression in lung tissue and low or null expression in normal LN. We consider molecular positive LN those in which expression of CEA or PLUNC was detected. In the 344 pathological negative LN, 13% (44/344) were positive for CEA, 16% (54/344) for PLUNC. The expression patterns were similar for both markers. At a median follow-up of 24 months (9−46) 11 p had died from NSCLC and 1 had died without recurrence. None of the living p had tumor recurrence. For the prognostic assessment, molecular positive LN were classified as N1 and N2. Median disease free survival was 15 ± 11.74 months in p with N2 molecular positive nodes and has not yet reached in cases of molecular negative LN ($p = 0.028$). Median survival of p with N2 molecular positive nodes was 17.3 ± 5.7 months and has not yet reached ($p = 0.0083$) for molecular negative LN.
Conclusions: CEA and PLUNC mRNA expression could be used as molecular markers of occult micrometastases in mediastinal LN showing a

prognostic effect. CEA and PLUNC expression provides a tool for selecting high-risk p considered for adjuvant therapies

6544 POSTER

Individualized high-dose continuous hyperfractionated accelerated radiotherapy (HI-chart) of non-small cell lung cancer (NSCLC) based on normal tissue constraints: a prospective clinical trial

A. van Baardwijk[1], L. Boersma[1], R. Wanders[1], A. Dingemans[2], G. Bootsma[3], W. Geraedts[4], C. Pitz[5], J. Simons[6], P. Lambin[1], D. De Ruysscher[1]. [1]*Department of Radiation Oncology (MAASTRO), GROW (Research Institute) University Hospital Maastricht, Maastricht, The Netherlands;* [2]*Department of Pulmonology, University Hospital Maastricht, Maastricht, The Netherlands;* [3]*Department of Pulmonology, Atrium Medical Centre, Heerlen, The Netherlands;* [4]*Department of Pulmonology, Maasland Hospital, Sittard, The Netherlands;* [5]*Department of Pulmonology, Sint Laurentius Hospital, Roermond, The Netherlands;* [6]*Department of Pulmonology, Sint Jans Gasthuis, Weert, The Netherlands*

Background: Local recurrence is a major problem after (chemo-)radiation for NSCLC. We hypothesized that for each individual patient the highest therapeutic ratio could be achieved by increasing the total tumor dose (TTD) to the limits of normal tissues, delivered within 5 weeks. In a theoretical model this resulted in an increase in tumor control probability from approximately 5% for a classical scheme (60 Gy in 6 weeks) to 25% for the study scheme. Here, we report the first results of a prospective clinical trial.
Materials and Methods: Twenty-nine patients with medically inoperable (stage I, n = 2) or locally advanced NSCLC (stage III, n = 27), in a good general condition (WHO-PS 0−1) and with a reasonable lung function (FEV1 >50% of predicted) were included. Most patients (25/29) received induction chemotherapy. All patients were irradiated using an individualized prescribed TTD, based on normal tissue constraints (mean lung dose 19 Gy, maximal spinal cord dose 54 Gy, no esophageal constraints) up to a maximal TTD of 79.2 Gy in 1.8 Gy fractions, twice daily. Toxicity was scored using the CTCAE-criteria. A FDG-PET-CT scan (n = 27) was performed to evaluate (metabolic) response 70 days after radiotherapy according to EORTC-criteria (PET) and RECIST-criteria (CT). The Kaplan-Meier method was used to compute overall survival.
Results: The mean delivered dose was 62.7 Gy (range 46.8−79.2 Gy), equivalent to a biological dose of approximately 80 Gy. Most patients experienced mild acute toxicity, while only 2 patients (7%) developed acute grade 3 toxicity (n = 1 dysphagia, n = 1 cough). Concerning late toxicity, 93% of patients (n = 25) showed radiographic changes (75% in <25% and 18% in >25% of the lungs), while 12 out of 28 patients (43%) had clinical symptoms (⩾gr 2 pneumonitis). One patient (3%) died 51 days after radiotherapy due to pneumonitis (treatment related mortality). The post-radiotherapy PET-CT showed in 18 patients a metabolic response (41% complete metabolic response, 26% partial metabolic response), whereas only in 9 patients (33%) a response was seen on CT (p = 0.01). Seventeen patients (59%) showed progressive disease, consisting of loco-regional progression (n = 6), metastases (n = 6) or a combination of both (n = 5). With a median FU of 16 months the median overall survival was 19.6 months and a 1-yr and 2-yr survival of resp. 59% and 45%.
Conclusions: Personalized HI-CHART radiation prescription based on normal tissue constraints is tolerable and initial results are promising.

6545 POSTER

Clinical significance of serum TERTmRNA detection in lung cancer patients

N. Miura[1], H. Nakamura[2], T. Harada[3], S. Takahashi[3], K. Shomori[4], H. Ito[4], Y. Adachi[2], J. Hasegawa[1], G. Shiota[5]. [1]*Tottori University, Pharmacotherapeutics, Yonago, Japan;* [2]*Tottori University, Thoracic Surgery, Yonago, Japan;* [3]*Tottori University, Pahrmacotherapeutics, Yonago, Japan;* [4]*Tottori University, Organ Pathology, Yonago, Japan;* [5]*Tottori University, Genetic Medicine and Regenerative Therapeutics, Yonago, Japan*

Background: Using a newly developed assay of telomerase reverse transcriptase (hTERT) mRNA in serum by real-time RT-PCR, we previously reported this assay to be superior to other tumor markers for hepatoma. In this study, we attempted to clarify its clinical significance as a biomarker for lung cancer.
Materials: In 89 patients with lung cancer and 27 individuals without it, we measured serum hTERT mRNA and epidermal growth factor receptor (EGFR) mRNA levels, using a quantitative one-step real-time RT-PCR assay. We examined its sensitivity and specificity in lung cancer diagnosis, its clinical significance in comparison with other tumor markers, and its correlation with the clinical parameters using multivariate analyses and correlation relative test.
Results: The copy number of serum hTERT mRNA was independently correlated with tumor size, tumor number, the presence of metastasis and recurrence, and smoking ($P < 0.05$, each). EGFR mRNA correlated with tumor size, tumor number, recurrence, and clinical stage ($P < 0.05$, each). The sensitivity/specificity in lung cancer diagnosis were 71.8%/72.5% for hTERT mRNA, 60.8%/62.5% for EGFR mRNA, respectively. hTERT mRNA was superior to other tumor markers in lung cancer diagnosis. Both mRNAs in serum were significantly correlated with those in lung cancer tissues ($P < 0.05$ for hTERT, $P < 0.05$ for EGFR, respectively). The copy number of hTERT mRNA significantly decreased after the surgical treatment.
Conclusions: The combination of both mRNAs improved the sensitivity/specificity to 82.8%/77.7%, thus suggesting that hTERT mRNA, especially when combining with EGFR mRNA, is a novel and excellent biomarker for pulmonary malignancies to diagnose and assess the clinical stage and effects of treatments.

6546 POSTER

Postoperative 3D conformal radiation therapy with dose-volume histogram assessment in non small-cell lung cancer

A. Zouhair[1], D. Dragusanu[1], O. Matzinger[1], B. Pehlivan[1], K. Khanfir[1], H.B. Ris[2], R. Stupp[3], R. Moeckli[1], R.O. Mirimanoff[1], M. Ozsahin[1]. [1]*University Hospital Center and University of Lausanne, Radiation Oncology, Lausanne, Switzerland;* [2]*University Hospital Center and University of Lausanne, Thoracic Surgery, Lausanne, Switzerland;* [3]*University Hospital Center and University of Lausanne, Medical Oncology, Lausanne, Switzerland*

Background: Despite many randomized trials, the indication of postoperative radiation therapy (PORT) in non small cell lung cancer (NSCLC) is controversial. Involved-field conformal (3D) RT has never been studied prospectively. In this study, we aim to assess the outcome of patients treated with involved-field 3D PORT with or without chemotherapy in locally advanced NSCLC.
Materials and Methods: From 1990 to 2006, data from 75 consecutive patients treated with curative surgery and PORT for NSCLC were retrospectively analyzed. Male to female ratio was 57/18, and median age was 58 years (38−76). There were 5 patients with stage I, 22 with stage II, and 48 with stage III disease. Pneumonectomy or lobectomy was realized in 24 and 51 patients, respectively. Mediastinal lymphadenectomy was performed in all patients. PORT indications were positive margins and/or positive mediastinal lymph nodes. Cisplatinbased chemotherapy was given in 15 patients. All patients had 3D conformal planning. Median RT dose was 60 Gy using at least 6-MV photons in 6 weeks, and CTV included bronchial stump and only positive nodal areas. Dose-volume histograms (DVH) assessing the pulmonary volume receiving 20 Gy (V20 Gy) were used in all patients.
Results: Compliance to PORT was 100%. In a median follow-up period of 55 months, 26 (35%) patients are alive without disease. Median overall survival time was 24 months, with survival rate of 35% at 5 years. The 5-year locoregional control and distant disease-free rates were 80% and 57%, respectively. Patients treated with pneumonectomy and those treated with at least 60-Gy PORT had better outcome. Grade 3 or more CTC v3.0 toxicity was observed only in 4 (5%) patients. No lethal toxicity was observed.
Conclusions: We conclude that involved-field 3D conformal 60-Gy PORT tailored with DVH V20 Gy assessment improves locoregional control without increasing lethal toxicity. Prospective studies using the above-mentioned criteria are warranted.

6547 POSTER

SNS-595: Preliminary results of 2 phase 2 second line studies in lung cancer

H. Burris[1], L. Krug[2], G. Shapiro[3], P. Fidias[4], J. Crawford[5], T. Reiman[6], G. Michelson[7], D. Young[8], D. Adelman[7], D. Ettinger[9]. [1]*Sara Cannon Cancer Center, Medical Oncology, Nashville TN, USA;* [2]*Memorial Sloan Kettering Cancer Center, Medical Oncology, New York NY, USA;* [3]*Dana Farber Cancer Institute, Medical Oncology, Boston MA, USA;* [4]*Mass General Hospital, Medical Oncology, Boston MA, USA;* [5]*Duke University Cancer Center, Medical Oncology, Durham NC, USA;* [6]*Cross Cancer Center, Medical Oncology, Edmonton AB, Canada;* [7]*Sunesis Pharmaceuticals Inc, Clinical Science, South San Francisco CA, USA;* [8]*Sunesis Pharmaceuticals Inc, Biometrics, South San Francisco CA, USA;* [9]*Johns Hopkins University, Medical Oncology, Baltimore MD, USA*

SNS-595 is a novel cell-cycle inhibitor that induces DNA damage responses, G2 arrest, and apoptosis. SNS-595 currently is being tested clinically in AML, ovarian cancer, and SCLC.