($p = 0.019$), CD25 ($p = 0.003$), CD127 ($p = 0.031$), Foxp3 ($p < 0.0001$) and TGF-β1 ($p < 0.0001$) between patients and controls. Pairmatched samples comparing pre and post-treatment expression of TGF-β1 showed that it was significantly reduced after chemotherapy. Additionally, patients with higher ratios (baseline/post-treatment) of CD4 and TGF-β1 were associated with local metastasis and progression, respectively. Survival analysis revealed that patients with combined high expression of CD25 and low expression of CD127 (reflecting a Treg phenotype), had significantly reduced TTP (median 2.40 months *vs* 5.47 months, $p = 0.001$) and a trend in OS (median 3.87 months *vs* 9.80 months, $p = 0.078$).

Conclusion: Based on gene expression analysis, it seems that the presence of a "Treg profile" in peripheral blood is associated with a poor prognosis in patients with advanced NSCLC.

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9132 POSTER

Efficacy Outcomes in First-line Treatment of Advanced NSCLC With Gefitinib (G) vs Carboplatin/paclitaxel (C/P) by Epidermal Growth Factor Receptor (EGFR) Gene-copy Number Score and by Most Common EGFR Mutation Subtypes − Exploratory Data From IPASS

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Background: IPASS (NCT00322452) demonstrated significantly improved progression-free survival (PFS) and objective response rate (ORR) with first-line G *v* C/P. EGFR mutation was a strong predictive biomarker for PFS benefit and tumour response to first-line G *v* C/P. PFS was prolonged for G *v* C/P in both common activating mutation subtypes. Here we report exploratory analyses of PFS and ORR in patients (pts) with high EGFR gene-copy number (high gene polysomy or gene amplification), and overall survival (OS) by most common EGFR activating mutation subtypes (Exon 19 deletion; L858R point mutation).

Methods: EGFR gene-copy number was determined by fluorescence in-situ hybridisation. High EGFR gene-copy number was defined as high gene polysomy (score 5; $\geqslant$4 copies in $\geqslant$40% of cells) or gene amplification (score 6; gene:chromosome $\geqslant$2, or $\geqslant$15 copies per cell in $\geqslant$10% cells). For each of these groups, hazard ratios (HRs; G:C/P) and 95% CIs were estimated for PFS using a Cox proportional hazards model adjusted for WHO PS (0, 1 *v* 2), smoking history (never *v* light ex-smoker) and gender. Odds ratios (ORs) and 95% CIs were estimated for ORR using a logistic regression model adjusted for the same covariates. EGFR mutations were detected using an amplification mutation refractory system with an EGFR detection kit. For pts with Exon 19 deletion or L858R mutation, HRs and 95% CIs were estimated for OS using a Cox proportional hazards model adjusted for the same covariates as PFS.

Results: 406 (of 1217 randomised) pts had known EGFR-gene-copy number biomarker status: 83 with gene amplification; 166 with high gene polysomy. PFS and ORR outcomes for G *v* C/P in pts with gene amplification: PFS HR 0.46, 95% CI 0.28–0.77; ORR OR 4.46, 95% CI 1.57–12.68; and in pts with high gene polysomy: PFS HR 0.77, 95% CI 0.53–1.11; ORR OR 1.46, 95% CI 0.79–2.71. Incidence of co-existing EGFR mutation was higher with gene amplification (86.7%) than high gene polysomy (71.1%). 261 pts had EGFR mutation-positive tumours: 140 with Exon 19 deletion; 111 with L858R. HRs for OS were 0.79 (95% CI 0.54–1.15) for Exon 19 deletion and 1.44 (95% CI 0.90–2.30) for L858R mutation.

Conclusions: PFS and ORR were improved with G *v* C/P in both gene-copy number score groups, with greater benefit with G in the gene amplification group. This was probably driven by an overlap with co-existing EGFR mutation, a known predictive biomarker for improved PFS and ORR with G in this setting.

9133 POSTER

Association Between TS, DHFR, and GARFT mRNA Expression and Efficacy of Pemetrexed in Advanced Non-small Cell Lung Cancr Patients

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Background: Pemetrexed (PMT), a multitargeted antifolate drug, inhibits three key folate enzymes: thymidylate synthetase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). PMT is effective in non-small-cell lung cancer (NSCLC) patients with non-squamous cell carcinoma. TS expression is lower in adenocarcinoma compared with squamous cell carcinoma. The relationship between clinical effectiveness of PMT and expression of folate enzymes in lung cancer cells is unknown. The purpose of this study is to determine whether TS, DHFR, and GARFT expression affect therapeutic efficacy of PMT.

Methods: The subjects were advanced NSCLC patients who treated with PMT. Samples were gotten by tumour biopsy before treatment. We dissected cancer cells from formalin-fixed paraffin-embedded tissues by using a laser microdissection. TS, DHFR, and GARFT mRNA were analyzed by using real-time RT-PCR. We assessed the association between TS, DHFR, and GARFT mRNA expression and therapeutic efficacy of PMT.

Results: Twenty-nine patients were enrolled. The median age was 67 years. Seventy-two percent of patients had a previous treatment with chemotherapy. Overall response rates were 27.6% for PMT. Median progression free survival (PFS) was 22.5 weeks for PMT. TS mRNA levels ranged from 0.001 to 33.590 (mean 2.451). TS mRNA expression was significantly lower in response group (CR+PR) compared with non-response group (SD+PD) (0.223 ± 0.083 versus 3.195 ± 1.752, $p < 0.001$). DHFR and GARFT mRNA expression were not correlated with response rate. PFS was superior for lower DHFR and GARFT mRNA expression patients compared with higher DHFR and GARFT mRNA expression patients, which was not statistically significant. (DHFR 29.1 versus 16.6 weeks, $p = 0.158$, GARFT 30.7 versus 16.6 weeks, $p = 0.071$).

Conclusions: We could analyze TS, DHFR, and GARFT mRNA expression in lung cancer cells specifically from biopsy specimens by using a laser microdissection. TS mRNA expression affected therapeutic efficacy of PMT. TS mRNA expression may be usefull predictive biomarker for NSCLC patients received PMT.

9134 POSTER

Tumour Response, Skin Rash and Health-related Quality of Life (HRQoL) − Post-hoc Data From the IPASS Study

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Background: IPASS (NCT00322452) demonstrated significantly longer progression-free survival (PFS) with first-line gefitinib *v* carboplatin/paclitaxel in never/light ex-smokers with advanced pulmonary adenocarcinoma in Asia, in the overall intent-to-treat (ITT) population and *EGFR* mutation-positive subgroup. We investigated objective response rate (ORR) and HRQoL in patients treated with gefitinib (ITT; *EGFR* mutation-positive subgroup) to further characterise the clinical relevance of the PFS data.

Methods: Objective response was assessed (RECIST) 6-weekly. Median time to response was summarised, median duration of response calculated (from first confirmed response visit) and change in tumour size assessed (percentage change from baseline) post-hoc. Patients without an end date were censored at their last evaluable assessment. The percentage of patients with a deterioration in HRQoL (reduction in Functional Assessment of Cancer Therapy-Lung [FACT-L; $\geqslant$6 points], Trial Outcome Index [TOI; $\geqslant$6 points]) or symptoms (Lung Cancer Subscale [LCS; $\geqslant$2 points]) at 4 months post-randomisation (median time on carboplatin/paclitaxel) was analysed according to progression status (post-hoc logistic regression adjusted for gender [male *v* female], WHO performance status [PS 0, 1 *v* 2] and smoking history [never *v* light ex-smoker]).

Results: In patients treated with gefitinib who responded, median time to response was 6.1 weeks (ITT; n = 262) and 6.0 weeks (evaluable *EGFR* mutation-positive subgroup; n = 94); median duration of response was 9.7 and 8.7 months, respectively. Change from baseline in tumour size demonstrated evidence of substantial tumour shrinkage with gefitinib (waterfall plots will be presented). Incidence of rash was 76% and 68% in *EGFR* mutation-positive and -negative subgroups, respectively (ORR 71% v 1%). Percentage of patients overall with a deterioration in HRQoL and symptoms at 4 months, progressors *v* non-progressors: FACT-L 33.7% *v* 16.3%, odds ratio (OR) 2.59, 95% CI, 1.54–4.34; TOI 33.7% *v* 13.2%, OR 3.34, 95% CI, 1.95–5.70; LCS 31.7% *v* 15.5%, OR 2.51, 95% CI, 1.48–4.26.
Conclusions: In IPASS, patients who responded to first-line gefitinib experienced substantial tumour shrinkage and a rapid, durable response. The data does not support rash as a predictive marker of response. A higher percentage of patients who progressed *v* non-progressors had a deterioration in HRQoL and symptoms.

9135 POSTER

Immune-modulating Effects of Bevacizumab and Metronomic Platinum Based-chemotherapy in Advanced Non-small-cell-lung Cancer (NSCLC) Patients

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Background: Bevacizumab is a humanized IgG1 mAb to the VEGF, with anti-angiogenetic activity, used for the treatment of different malignancies, including NSCLC. VEGF exerts multiple functional activities throughout the binding to three receptors expressed on different cell lineages, including endothelial precursors, CNS neurons, myeloid precursors, dendritic cells (DCs) and lymphocytes. We investigated the immune-modulating effects of bevacizumab in advanced NSCLC patients enrolled in the mPEBev phase I-II trial, who had received frontline biochemotherapy with cisplatin, oral metronomic etoposide and bevacizumab (mPEBev regimen).
Patients and Methods: Forty-eight patients (42 males and 6 females) with stage IIIB/IV NSCLC, and ECOG $\leqslant$ 2 were enrolled in this study (EUDRACT code #BEVA2007) and received iv. cisplatinum (30 mg/sqm, days 1−3), oral etoposide (50 mg, days 1−15) and bevacizumab (at dosage of 0; 2.5; 5; 7.5; and 10 mg/kg, day 3), every three weeks. We carried-out an immunological analysis on the PBMCs and serum of these patients taken at baseline and after 4 treatment courses.
Results: This regimen resulted moderately safe and very active with 66.7% (32 patients) response rate, 9 month median progression-free-survival, and 35% survival at 15 months. Our biological monitoring revealed a progressive decrease in monocyte and neutrophil counts, associated with myeloperoxidase activity decline [2133.3 ($\pm$162.2) vs 1413.2 ($\pm$110.05), P = 0.044]. Flow cytometry revealed a significant treatment-related decrease of inhibitory-myeloid cells and an increase of activated DCs ($CD11c^+CD14^+CD80^+CD83^+$) (2.48% vs 5.48%, P = 0.03) in patients' PBMCs, which were associated to a decline of the absolute number of peripheral $CD3^+CD4^+$ T cells, expressing an early memory ($CD3^+CD27^+$) and immune-suppressive regulatory ($CD4^+CD25^+FoxP3^+$) (T_{reg}) immune-phenotype. It was finally, observed an increase in activated cytotoxic-T-cell (CTL) effector ($CD3^+CD62L^+CD8^+$) number. In these patients there was a substantial decrease of Interleukin-10 levels [325.9 ($\pm$54) vs 187.5 ($\pm$24) ng/ml, P < 0.05] indicating an immunologic cytotoxic Th1-phenotype switch and impairment of inhibitory myeloid cells.
Conclusion: This bevacizumab-based regimen exerts immune-modulating effects in NSCLC patients, resulting in a major neutrophil and inhibitory-myeloid cell impairment, associated with a significant increase in activated DCs and CTLs able to trigger an efficient immune-response with potential anti-tumour activity.

9136 POSTER

Can Serum Be Used for Analyzing the EGFR Mutation Status in Patients With Advanced Non-Small Cell Cancer (NSCLC)?

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Background: Epidermal growth factor receptor (EGFR) mutations as prognostic or predictive marker in patients with non-small cell cancer (NSCLC) have been used widely. However, it may be difficult to get tumour tissue for analyzing the status of EGFR mutation status in large proportion of patients with advanced disease.
Patients and Methods: We obtained pairs of tumour and serum samples from 57 patients with advanced NSCLC, between March 2006 and January 2009. EGFR mutation status from tumour samples was analyzed by genomic polymerase chain reaction and direct sequence and EGFR mutation status from serum samples was determined by the peptide nucleic acid locked nucleic acid (PNA-LNA) PCR clamp.
Results: EGFR mutations were detected in the serum samples of 11 patients and in the tumour samples of 12 patients. EGFR mutation status in the serum and tumour samples was consistent in 50 (87.7%) of the 57 pairs. There was a high correlation between the mutations detected in serum sample and the mutations detected in the matched tumour sample (correlation index; 0.62 P < 0.001). Twenty-two of 57 patients (38.5%) received EGFR-TKIs as any line therapy. The response for EGFR-TKIs was significantly associated with EGFR mutations in both tumour samples and serum samples (p < 0.05). There was no significant differences in OS according to the status of EGFR mutations in both serum and tumour samples (p > 0.05).
Conclusion: Serum sample might be alternatively used in the difficult time of getting tumour tissue for analyzing the status of EGFR mutation status in patients with advanced NSCLC.

9137 POSTER

NF-kappaB Activation and Distinct Expression of Ubiquitin E3 Ligases in Skeletal Muscle of Patients With Non-small Cell Lung Cancer Cachexia

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Background: Clinical oncologists recently emphasized the need for attention of cancer cachexia and muscle wasting in clinical oncology because it decreases response to anticancer therapy and increases morbidity and mortality. Experimental research has shown that increased muscular Nuclear Factor kappa B (NF-κB) and subsequent ubiquitin (Ub) proteasome system (UPS) activity plays a causal role in cancer-induced muscle wasting but this needs verification in human cancer cachexia. The aim of this study was to investigate molecular changes (including NF-κB activity and expression of E3 Ub-ligases) in skeletal muscle of cachectic patients with advanced NSCLC.
Methods: In this prospective study, 14 cachectic patients (defined by >5% body weight loss in preceding 6 months) and 12 non-cachectic patients with newly diagnosed advanced stage NSCLC and 22 age and gender matched healthy controls were included. Body composition was assessed by dual energy X-ray absorptiometry (DXA) and biopsies were obtained from vastus lateralis muscle. IkappaBalpha (IκBα) and TNF-α mRNA expression levels were determined as indirect indices of NF-κB activity. UPS activity was evaluated by mRNA expression of E3 Ub-ligases: neural precursor cell expressed developmentally down-regulated 4 (NEDD4), Atrogin-1, Tripartite motif-containing protein 32 (TRIM32) and Muscle RING-finger protein-1 (MuRF1).
Results: Mean weight loss was 13.1$\pm$4.9% in cachectic patients compared with 2.1$\pm$2.0% in the non-cachectic group. Cachectic patients had significantly decreased skeletal muscle mass content of upper and lower extremities compared with non-cachectic patients (p = 0.02) and healthy controls (p < 0.001).
Muscle IκBα (p = 0.04) and TNF-α (p = 0.03) mRNA expression and NEDD4 (p = 0.03) were significantly increased in cachectic patients compared with healthy controls. No increase was observed in the other E3 Ub ligases. In contrast, TRIM32 (p < 0.01) showed a significant decrease in non-cachectic patients compared to both cachectic patients and healthy controls, whereas MURF-1 levels were unchanged.
Conclusions: These results indicate that NF-κB activity is increased in lung cancer cachexia but show that E3 Ub-ligases are expressed differently. As all assessed E3 Ub-ligases have shown to be important regulators in experimental models of cachexia, the observed distinct regulation in cachectic patients with NSCLC has implications for understanding the mechanism of human lung cancer cachexia. Further research is required to investigate the potential contribution of individual E3 Ub-ligases to human cancer cachexia in order to develop therapeutic interventions to prevent or reverse cachexia and increase survival of lung cancer patients.