with poliacrilamid gel stained with silver nitrate. Gel was scanned and analysed with *Progenesis* PG6220 program, which measures intensity of each spot. Resultant intensities in each group of patients (SCLC/non pathological bronchoscopy) were compared using T-Student method. We selected as potential markers those spots with a *p* value of less than 0.05. We calculated "fold change" of each spot as the ratio between mean intensity in SCLC samples and non pathological samples.
**Results:** Optimal bidimensional gels of each sample were obtained. Among 300 comparable spots, 10 of them were expressed with a different intensity in both groups of patients; 6 of these potential markers were over expressed in SCLC samples, whereas 4 of them were under expressed. The "fold change" of these 10 spots ranges from 1.5 to 8.67.
**Conclusions:** Different protein markers can be detected in bronchial fluid obtained from SCLC samples. Significant differences in expression of these biomarkers were detected between SCLC patients and non pathological bronchoscopy patients. The development of an early diagnostic test using these proteins must be validated in future studies.

**1342** POSTER
**NBL1 and anillin (ANLN) genes expression as diagnostic markers for pancreatic carcinoma**

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Diagnostic approach to pancreatic tumors is very often limited by the effectiveness of thin-needle biopsy to confirm the malignancy. Thus, search for novel markers specific for pancreatic cancer is highly substantiated. Based on our microarray study, we have chosen to validate two candidate genes, first indicated by a landmark papers of Iacobuzzio-Donahue et al. and not evaluated further for their association with pancreatic cancer.
The aim of our the study was to verify the utility of gene expression of NBL1 (Neuroblastoma, suppression of tumorigenicity 1) and ANLN (Anillin), as novel molecular markers of pancreatic cancer.
**Material and Methods:** Initial part of study was based on microarray analysis of 18 pancreatic adenocarcinomas and 16 benign samples (9 chronic pancreatitis specimens and 7 from grossly normal pancreas), by HG-U133 Plus 2.0 oligonucleotide Affymetrix arrays. The obtained dataset was pre-processed using GC-RMA method, gene expression values were compared by parametric t-test with False Discovery Rate estimated by Benjamini-Hochberg method. Validation part of the study was carried out in 66 samples: 31 adenocarcinomas and 35 benign specimens (21 samples of normal pancreas and 14 chronic pancreatitis). Real-time quantitative PCR reaction was performed in all validation set samples on Applied Biosystems SDS 7700 machine with Universal Probe Library fluorescent probes (Roche). We analyzed four reference genes: ATP6V1E1, EIF3S10, HADHA, UBE2D2 and normalized the obtained result to the reference index obtained by geNorm software.
Based on microarray data, most pronounced difference was observed for NBL1 gene, with 34.7-fold increase of expression in cancer. This was confirmed by validation study, where NBL1 gene was 9.5-fold over-expressed in cancer vs normal samples. For ANLN gene a gradual increase in expression from normal samples by chronic pancreatitis to large values in pancreatic cancer was observed (cancer/normal 19.8-fold, cancer/pancreatitis 4.0-fold). For both genes we confirmed the statistically significant differences in gene expression between pancreatic cancer, chronic pancreatitis and normal pancreas ($p < 0.0001$, Kruskall-Wallis ANOVA). In post-hoc inter-group comparisons, both genes differentiated between cancer/normal ($p < 0.000001$) and cancer/pancreatitis ($p < 0.000001$ for ANLN and $p = 0.000001$ for NBL1). By ROC curve analysis we showed that combining both markers gives a significant increase in classification accuracy.
**Conclusion:** NBL1 and anillin are promising markers for pancreatic carcinoma molecular diagnostics.

# Radiotherapy and radiobiology

## *Oral presentations* (Thu, 24 Sep, 09:00–11:00) Radiotherapy and radiobiology

**2000** ORAL
**Clinical validation of atlas-based auto-contours in the head & neck**

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**Objective(s):** For optimal sparing conditions in the H&N, one requires accurate delineation of target volumes and critical normal tissues. For that purpose a CT-based- atlas of the neck levels I-V and guidelines or critical OAR, were developed. Contouring is tedious, time consuming and suffers from large intra- and inter-observer variability's. A promising new tool is Auto-Contouring (AC) by multiple-subject Atlas-Based Auto-Segmentation (ABAS, CMS, Inc.) of CT-images. Our preliminary results with ABAS, validates the accuracy of the delineation process with or without the help of AC, and we present our analyses the amount of reduction in contouring time that can be realized through ABAS.
**Materials/Methods:** Eleven N0/N+ patients were selected. In all patients the neck levels I-V (both necks), and 19 OARs were contoured by two staff members; total contouring times were recorded. These reference contours were regarded as the gold standard and used as input for ABAS. In 4 of these patients the generated AC were edited by the 2 staff members and editing times were recorded. Next, for 12 clinically IMRT treated patients, 5 experienced observers edited the generated auto-contours (on average 4 patients per observer). In all cases the neck node levels and the 19 defined OARs were auto-contoured and editing times were recorded. Dice coefficients (0 indicates no overlap, 1 a perfect agreement) were calculated to quantify the similarity to the gold standard of the clinically contoured-, the auto-contoured- and the edited structures. Finally, an expert panel scored all AC contours as well as the edited AC contours regarding their adequacy relative to the Atlas: 0 = poor, 1 = moderate, 2 = good. For AC the following scoring system was used: 0 = poor, 1 = major deviation, editable, 2 = minor deviation, editable, 3 = perfect.
**Results:** The initial contouring time was 180 minutes per patient on average; editing times approximately 39 minutes. The mean Dice coefficients of the AC contours vs. clinically used delineations were 0.7/0.8/0.8 for the neck node levels, parotids, and submandibular glands, respectively. For the AC contours versus the edited AC contours the Dice were 0.8/0.9/0.8. The expert panel scored 100% of the AC of the neck levels as a minor-deviation-editable or better. The expert panel scored 88% of the edited contours as good, where 83% of the clinically used contours were scored as good.
**Conclusions:** Multiple-subject ABAS of CT images proved to be a useful novel tool in rapid delineation of normal and target tissues. Although editing of the auto-contours is inevitable (39 min), substantial time reduction was achieved by editing instead of contouring from scratch (180 vs. 39 min.). This is even more relevant since the edited contours were of similar or better quality than the clinical ones.

**2001** ORAL
**Acute toxicity of curative radiotherapy for intermediate risk localized prostate cancer in the EORTC trial 22991**

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**Introduction:** EORTC trial 22991 randomly assessed the addition of concomitant and adjuvant short-term hormonal therapy to curative conformal/intensity-modulated radiotherapy (RT) for intermediate risk localized prostate cancer. We report the acute toxicity (assessed weekly during RT) for the organs at risk (genito-urinary (GU) and gastro-intestinal (GI)) in relation to radiation parameters.