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.

Material and Methods: Eligibility criteria were age ⩽80 years, PSA ⩽ 50 ng/ml, N0M0 and either tumour stage cT2a (1997 UICC TNM) or cT1b-c combined with PSA⩾10 ng/ml and/or Gleason score ⩾7.
We report toxicity for all eligible patients who received the planned RT with documented acute toxicity (CTCAEv.2) and RT-quality assurance parameters. The RT dose (70 Gy, 74 Gy or 78 Gy) and technique (3D-CRT vs IRMT) were per institution choice, the randomization was stratified for institution. Statistical significance was set at 0.05. (ClinicalTrials.gov: NCT00021450)
Results: Of 819 randomized patients, 28 were excluded from the analysis (3 with <60 Gy RT, 25 with missing information). Of the 791 analysed patients, 652 (82.4%) were treated with 3D-CRT, 139 with IMRT. In the 3D-CRT group, 195 patients (29.9%) were treated with a total prescribed dose of 70 Gy; 376 (57.7%) with 74 Gy and 81 (12.4%) with 78 Gy. In the IMRT group, 28 (20.1%) were treated to a total dose of 74 Gy and 111 (79.9%) with 78 Gy.
Overall, only 7 of 791 patients (0.9%) had grade 3 GI toxicity during RT: diarrhea (N = 6), rectal bleeding (N = 1) and proctitis (N = 1). Fifty patients (6.3%) had grade 3 GU toxicity: urinary frequency (N = 38, 4.6%), dysuria (N = 14, 1.7%), urinary retention (N = 11, 1.3%), urinary incontinence (N = 2) and hematuria (N = 1). No grade 4 toxicity was reported. Hormonal treatment did not influence the risk of side effects (p>0.05). The risk of grade ⩾2 GI toxicity significantly correlated to D50%-rectum (p = 0.004) with a cut-of value of 44 Gy. The risk of grade ⩾2 GU toxicity was moderately affected by Dmax-bladder (p = 0.051). Overall, only 14 patients (1.8%) had residual grade 3 toxicities one month after RT.
Conclusion: 3D-CRT and IMRT up to 78 Gy is well tolerated. Dmax-bladder and D50%-rectum were related to the risk of grade⩾2 GU and GI toxicity, respectively. IMRT lowered D50% rectum and Dmax-bladder. An irradiated volume >400 cc for 3D-RT and a dose of 78 Gy, even for IMRT, negatively affected those parameters and increased the risk for toxicity.

2002 ORAL

Development and external validation of a nomogram for prediction of radiation-induced dysphagia in 493 lung cancer patients treated with chemo-radiotherapy or radiotherapy alone

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Background: Acute dysphagia is a distressing dose-limiting toxicity occurring frequently during concurrent chemo-radiation or high-dose radiotherapy for lung cancer. It can lead to treatment interruptions and subsequently jeopardize tumor control. Although a number of predictive factors associated with dysphagia have been identified it is still not clear how these factors should be used in daily clinical practice and how they could offer assistance for treatment decision making. We have therefore developed and validated a nomogram to predict acute severe dysphagia in lung cancer patients who are receiving (chemo)radiotherapy.
Material and Methods: Clinical data from 493 lung cancer patients, treated with curative intent with chemo-radiation (CT-RT) or radiotherapy (RT) alone were collected. An ordinal regression analysis was performed to predict severe acute dysphagia (according to the CTCAEv3.0). The start model consisted of age, gender, World Health Organisation performance status (WHO-PS), mean esophagus dose, maximum esophagus dose, overall treatment time (OTT), radiation once (QD) or twice daily (BID) and chemotherapy. Odds ratios (OR) were reported. The final model was validated using bootstrap techniques as well as an external dataset from Ghent University (n = 117). The performance of the model was expressed as the C-statistic. The interpretation is similar to the interpretation of the Area Under the Curve (AUC) of the Receiver Operator Curve (ROC). The maximum value of the C-statistic is 1.0; indicating a perfect prediction model. A value of 0.5 indicates that patients are correctly classified in 50% of the cases, e.g. as good as chance. In addition, the results of the multivariate analysis were used to develop a nomogram.
Results: Fifty-three patients (11%) developed acute severe dysphagia ⩾grade III. The final model consisted of mean esophagus dose (OR 1.06, p < 0.0001), maximal esophagus dose (OR 1.04, p < 0.0001), OTT (OR 0.94, p < 0.0001), chemotherapy treatment (OR sequential 1.02, OR concurrent 2.86; p < 0.0001) and gender (female OR 1.99; p = 0.0003). The C-statistic was 0.77 (0.76 internal validation). External validation using the dataset from Ghent yielded a C-statistic of 0.86.
Conclusions: The performance of the model, C-statistic of 0.76, was good. In addition, external validation yielded a C-statistic of 0.86 and was thus successful. The nomogram could be used in clinical practice to identify patients at high risk for developing severe acute dysphagia.

2003 ORAL

Angiotensin converting enzyme inhibitors (ACE-I) protect against the clinical and radiological manifestations of radiation pneumonitis (RP) in patients undergoing radical radiotherapy for lung cancer

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Background: Previous laboratory investigations have highlighted a possible role of the renin angiotensin system in the pathogenesis of RP. ACE-I have also been shown to reduce radiation pneumotoxicity in animal studies. However a previous clinical study, which predates modern 3-dimensional treatment techniques, did not find an association between these agents and the development of RP.
Material and Methods: The records of 145 patients (pts) who had undergone radiotherapy for stage I-III NSCLC were reviewed. Median age 70, male:female 102:43, induction chemotherapy administered in 52. All pts were treated with a single radiotherapy dose, 54 Gy in 36 fractions over 12 days (CHART). 3-dimensional conformal treatment was used in all cases. Medications taken at the time of treatment were noted. 19 pts were taking ACE-I for hypertension or congestive cardiac failure. The development of RP was recorded prospectively using the RTOG criteria. All pts had a diagnostic CT scan performed 3 months following completion of RT. Radiation induced radiographic abnormalities within the lung were graded using the system of Libshitz and Shuman (G0 = no change, G1 = 'ground glass' changes, G2 = patchy consolidation, G3 = confluent consolidation)
Results: In total 24 (17%) pts developed clinical RP ⩾G3 (requiring steroids). Radiographic intrapulmonary abnormalities were seen in 92 (63%). These were grade as: G1 – 15 (10%), G2 – 19 (13%) and G3 – 58 (40%). The association of lung injury and medication with ACE-I is shown in the table.

	ACE-I	No ACE-I	p-value
Clinical RP ⩾G2	0/19 (0%)	24/126 (19%)	0.037
Radiological injury ⩾G2	2/19 (11%)	75/126 (60%)	<0.001
V_{20} (mean)	19.1%	20.9%	0.33
Mean lung dose (mean)	10.6 Gy	11.4 Gy	0.37

Conclusions: In this series of pts treated in the modern era, ACE-I appear to offer significant protection against radiation induced lung injury. Their use as pulmonary radioprotectants warrants further investigation. The results of the randomised RTOG trial (0123) are awaited with interest.

2004 ORAL

Intraperitoneal delivery of Chitosan/siRNA nanoparticles targeting TNF-a prevents radiation-induced fibrosis

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Introduction: Ionizing radiation therapy plays a pivotal role in cancer treatment but one of the most common long-term adverse effects of ionizing radiotherapy is radiation-induced fibrosis (RIF). Recent studies suggested Tumor Necrosis Factor (TNF-a), produced by macrophages might promote radiation-induced fibrosis.
This work describes intraperitoneal delivery of chitosan/siRNA nanoparticles targeting TNF-a as prevention for radiation-induced fibrosis. CDF1 mice treated with TNF-a siRNA at least until day 22 after radiation did not develop fibrosis whereas the control groups treated either with buffer or mismatch siRNA develop severe fibrosis.
Experimental Methods: Chitosan was dissolved in sodium acetate buffer (0.2 M NaAc, pH 4.5) to obtain a 1 mg/ml solution and then adjusted to pH 5.5. 20 μl of siRNA (100 μm) in nuclease free water was added to 1 ml of filtered chitosan (1000 μg/ml) whilst stirring and left for 1 h.
Male CDF1 mice were divided into 9 groups of 3. Except of the control group with no treatment, all mice received a single irradiation dose of 45 Gy. Mice were i.p. dosed with 200 μl of chitosan/siRNA nanoparticles (5 μg TNF-a siRNA and 5 μg mismatch siRNA) 2 days before irradiation or 1 day after irradiation. The chitosan/siRNA nanoparticles treatment was continued twice a week and terminated on days 10, 22, 34 and 258.
The irradiated hind leg of the mice was scored for clinical symptoms of radiation-induced fibrosis by using the leg contracture model.
Results: The irradiated hind leg of the mice was scored for clinical symptoms of radiation-induced fibrosis by using the leg contracture model for level of severe fibrosis (scale: 0 = normal, 1−2 = mild to moderate