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

fibrosis, 3−4 = severe fibrosis). The development of severe fibrosis is dramatically higher in the combined control groups (N = 14) compared to the group of animals treated with TNF-a siRNA for 22 or more days (P = 0.00003). Interestingly, mice dosed with chitosan/siRNA only until day 10 developed fibrosis. We hypothesized that prevention of radiation-induced fibrosis is linked to the duration of administration and therefore a successful therapy against RIF is only given if chitosan/siRNA nanoparticles have been administered until say 22 or longer.
Conclusion: This study describes a novel strategy to prevent radiation-induced fibrosis by targeting TNF-a knockdown in systemic macrophages.

2005 ORAL

Die hard? Radiation sensitivity of cancer stem cells from established human cell lines

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Background: Cancer stem cells (CSC) are postulated to mediate tumour radiaton resistance and late relapse. Recently developed technologies to isolate cells expressing specific surface markers of CSC allow to study the radiobiological properties with functional assays in cell lines where CSC where CSC were postulated to represent a subpopuolation. We aimed to identify CSC in a panel of eight cell lines from different organs and test their radiobiological differences.
Materials and Methods: Cell lines were stained with specific CSC antibodies and sorted into CSC populations with FACS or magnetic beads. Sorted and unsorted populations were analyzed for γH2AX foci and radiosensitivity. CSC phenotype was confirmed with anchorage indepent growth test and activated Notch1 immunoblotting. All in vitro experiments were performed in both chemically defined low growth factor containing media and serum containing media. Xenograft tumours were treated with fractioned radiation to test selection for CSC and tumourigenicity was tested in SCID mice.
Results: CSC specific surface markers were detected in all of the tested cell lines in good agreement with evidence from primary tumours of the tested tumour types. The CSC fractions of the breast cancer cell line MDA-MB-231, and pancreatic cancer cell lines Panc-1 and PSN-1 all had less residual γ-H2AX foci compared to the unsorted cell lines pointing to radiation resistance of CSC. However, only MDA-MB-231 CSC and none of the other cell lines CSC had increased postradiation clonogenic survival compared to unsorted cells. Enhanced anchorage independent growth in MDA-MB-231 but not in PSN-1 and over expression of activated Notch1 confirmed the CSC phenotype of MDA-MB-231 and PSN-1 subpopulations. Notch1 expression was also enhanced in PSN-1 and Panc-1. The expression of surface markers in MDA-MB-231 was shifted to a CSC-type pattern after fractionated radiation und xenograft tumourigenicity was enhanced in MDA-MB231 but not in PSN-1 CSC subpopulations.
Conclusions: Although we reliably identified subpopulations expressing previously described organ type specific CSC surface markers in cell lines we could not confirm the radioresistant phenotype in this model in general. This is critical to consider in exploring models essential for assessing the biological advantage of CSC.

2006 ORAL

Local tumour control after simultaneous fractionated irradiation and EGFR-blockade by monoclonal antibodies (Cetuximab) versus tyrosine kinase inhibitors (Erlotinib) in different head and neck squamous cell carcinoma (HNSCC) models

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Background: A wide variability of response to EGFR inhibition and radiotherapy has been observed between different tumours but also between different classes of drugs. Here, potential mechanisms of this heterogeneity are evaluated.
Material and Methods: The effect of radiotherapy alone (30 fractions/6 weeks) or with simultaneous EGFR inhibition by the antibody cetuximab versus the TK inhibitor erlotinib is compared in different HNSCC xenograft models. Endpoint is permanent local tumour control, measured tumour control dose 50% (TCD50) for the irradiation arms and tumour growth delay for the drugs alone. Immunohistochemical (IHC)/immunoflourescence (IF) techniques are used for proliferation/micromilieu, western blots for expression/phosphorylation of receptors/downstream molecules.
Results: Preliminary data on the first tumour models, UT-SCC-5 (ELISA: EGFR-low) and SAS (EGFR-moderate), both expressing no mutations of the EGFR-TK binding domain or of KRAS, are available. TCD50 values are listed below. In UT-SCC-5, local tumour control was not different after irradiation alone or combination with erlotinib or cetuximab. Tumour growth delay was not influenced by the drugs alone, but slightly prolonged after combined treatment in some irradiation dose groups. In SAS tumours, cetuximab significantly improved local tumour control, whereas erlotinib tends to impair local control. IHC/IF evaluations and western blot data after 6 treatment days are currently available for UT-SCC-5. Briefly, a slight reduction of S-phase after combined irradiation and Erlotinib was observed, but no effect in the other groups or on Ki67 (proliferation) and Pimonadizole (hypoxia). Total EGFR and ErbB2 decreased in both Cetuximab arms, Erlotinib in both arms decreased phosphorylation of ErbB2 and, when given alone, decreased MAPK phosphorylation.
Conclusion: Local control of UT-SCC-5 tumours after fractionated irradiation was not improved by simultaneous cetuximab or erlotinib treatment, whereas in SAS tumours cetuximab significantly improved local control and Erlotinib tended to impair local control. Western blot and IHC/IF data of both tumour models are underway and will be presented.

	TCD50 (Gy) [95% C.I.], p-values vs. irradiation alone		
	Irradiation alone	irradiation + cetuximab	irradiation + Erlotinib
UT-SCC-5	111.9 [97; 128]	119.5 [101.2; 159.1], n.s.	103.4 [93; 117], n.s.
SAS	110.6 [98; 126]	76.3 [63; 89], p = 0.001	129.7 [112; 160], p = 0.06

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2007 ORAL

First report on the patient database of the identification of the genetic pathways involved in patients overreacting to radiotherapy: GENEPI-II

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Background: For radiotherapy, a dose-response relationship has been found, implying that higher doses also lead to higher tumor control rates. This is hampered by normal tissue toxicity. However, as the incidence of severe, late irreversible tissue damage could not exceed 5%, the 5% most radiosensitive patients thus determine the prescribed radiation doses. Identifying the most radiosensitive group would therefore have huge clinical implications.
Methods: A tissue bank containing skin fibroblasts, whole blood, lymphocytes, plasma and lymphoblastoid cell lines from clinically radiation hypersensitive patients was established from patients in Europe and Canada. A control group of patients, namely those who do *not* exhibit abnormal reactions to radiotherapy is already available from the GENEPI I study. Overreacting individuals (CTCAE3.0 severe acute side effects grade **2 or more** occurring at very low radiation doses where these side effects are unexpected or grade **3−4** lasting more than 4 weeks after the end of radiotherapy and/or requiring surgical intervention at any time; severe late side effects grade **3−4** occurring or persisting more than 90 days after the end of radiotherapy) excluding known hypersensitivity syndromes, had to exhibit severe acute **or** late side effects after radiotherapy **without** concurrent chemotherapy, biologicals, targeted drugs or radio-protectors at doses from which these side effects are reported to occur in less than 1/500 patients. 3D radiation dose distribution should be known and dosimetry checks are included.
Results: At present, 33 patients have been identified: 10 males and 23 females. Patient groups include breast (15), prostate (5), cervix (4), head and neck (3), lymphoma (3), endometrium (1), lung (1) cancer and medulloblastoma (1). The mean age was 56.6±15.2 years (S.D.) (range 3−78). The radiation dose was 49.3±17.6 Gy (15−90). The mean time to develop severe side effects after radiotherapy was 675±40.3 days (0–2705). 8/33 (28.6%) experienced severe acute side effects, the other 25 patients late damage. Severe side effects included acute skin