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.

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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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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