(ASM) was shown to be relevant for radiation induced cell death in endothelial cells. In turn, side effects at the bowel and in the brain which are at least partially mediated by endothelial alterations may be ameliorated by inhibition of ASM mediated ceramide production. Basic fibroblast growth factor clearly reduces the ceramide production via ASM and was shown to interfere with radiation induces side effects in the brain and the bowel in murine test systems.

Conclusion: Although the examples presented above cannot be considered to reflect all available strategies aiming on the modulation of cell death pathways, it becomes clear that several promising approaches targeting defined cell death pathways have been developed. The use of ALPs in a phase I trial is one of the first important examples proving that basic research in radiation biology finally guides the development of new treatment strategies. This and other approaches will hopefully increase tumor control rates and reduce side effects in the future.

250 NON-INVASIVE EVALUATIONS OF BACTERIA-BASED SUICIDE GENE TRANSFER: PRE-CLINICAL *IN VITRO* AND *IN VIVO* SCREENING USING MR SPECTROSCOPY

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Objective: Clinical monitoring of gene therapy ultimately necessitates non-invasive approaches to eventually allow for modification and optimization on an individual basis. We are investigating the potential of bacteria-mediated vector systems based on the use of non-pathogenic *Clostridium* and *Salmonella* species for the tumour-specific transfer of cytosine deaminase (e.g. Theys et al 2001) which, in combination with systemic delivery of 5-Fluorocytosine (5-FC), is expected to result in anti-tumour effects. The present research aimed to apply ^{19}F-MR Spectroscopy (^{19}F-MRS) and to evaluate the *in vivo* feasibility for detecting 5-Fluorouracil (5-FU) in rodent tumours selectively colonized with CDase-recombinant non-pathogenic bacteria.

Materials and Methods: Non-pathogenic *Clostridium acetobutylicum* DSM792 and attenuated *Salmonella*Typhimurium VNP20047 (VION Pharmaceuticals), both recombinant for CDase were tested for 5-FC to 5-FU conversion. The *in vitro* ^{19}F-MRS measurements were done on bacterial lysates (overnight incubation with 5-FC) as well as on tissue extracts (tumour and liver) from bacteria-treated animals, using a high resolution AMX 360 (8.4 Tesla) spectrometer (Bruker). *In vivo*, the intra-tumour conversion activity of the recombinant bacteria was evaluated in a 4.7 Tesla BIOSPEC horizontal magnet (Bruker), equipped with a double-tuned (^{19}F-^{1}H) surface coil of 1 cm diameter, positioned underneath the tumour. Animals were anaesthetized with Nembutal^{R} for stable positioning. Serial ^{19}F spectra were acquired every 15 minutes, while the animal environment was kept at 37°C. Subcutaneous xenografted (nu/nu mouse) human colon carcinoma (HCT116 and HT29) and the syngeneic WAG/Rij rat rhabdomyosarcoma were used. 5-FC was administered intraperitoneally and/or intratumourally.

Results: The expression of functional CDase by attenuated *S.*Typhimurium VNP20047 and by *C. acetobutylicum* DSM792 was demonstrated *in vitro* by MR Spectroscopy of bacterial lysates. Conversion of 5-FC to 5-FU (chemical shift difference of 1.2-1.3 ppm) was found to correlate with bacterial expansion (optical density of suspension at analysis). This technique allowed a more secure and sensitive measurement of 5-FU conversion as possible with thin layer chromatography. Extracts of tumours from CDase-recombinant bacteria *plus* 5-FC treated animals also clearly showed the production of 5-FU with the ^{19}F-MRS *in vitro* investigation. Non-toxic catabolites were only slightly detectable in liver extracts, indicating the poor diffusion of 5-FU into the systemic circulation. The various experiments have been proven very reproducible. Pilot experiments involving the *in vivo* ^{19}F-MRS evaluation of bacterial CDase activity demonstrated the sensitivity and accuracy of this non-invasive technique to detect intratumour 5-FC and 5-FU. With the different 5-FC administration routes, conversion to 5-FU was seen upon the analysis of the spectra. The area-under-the-curve of the 5-FU correlated with the length of the time interval between injection of CDase-recombinant bacteria and ^{19}F-MRS analysis (colonization quality), and likely with the 5-FC concentration available in the tumour.

Conclusion: The potential applicability to use ^{19}F-MRS for longitudinal non-invasive screening of this bacteria-based suicide gene transfer system.

251 PROSPECTIVE STUDY OF CD4 AND CD8 T-LYMPHOCYTE APOPTOSIS AS A MARKER FOR RADIATION-INDUCED LATE EFFECTS IN 399 INDIVIDUAL PATIENTS

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Objective: Previous retrospective studies suggested that low radiation-induced T-lymphocyte apoptosis could be correlated with late normal tissue toxicity (Crompton *et al*; IJROBP 1999, 45:707-714 and IJROBP 2001, 49:547-554). In the present study, we wanted to assess prospectively the usefulness of radiation-induced CD4 and CD8 T-lymphocyte apoptosis in the prediction of individual intrinsic radiosensitivity.

Material and Methods: Between 1998 and 2000, 399 consenting patients with miscellaneous cancers treated by curative radiation therapy (> 50 Gy) were included in the KFS 00539-9-1997/SKL 00778-2-1999 study. All patients were tested using a rapid (48 h) leukocyte apoptosis assay, where fresh blood samples were irradiated with 8-Gy X rays. Following irradiation, lymphocytes were collected and prepared for flow cytometric analysis. Cytotoxicity was assessed by gradual degradation of internucleosomal DNA which resulted in a sub-G1 peak on the DNA histogram. The majority of the patients had breast (n = 149), head and neck (n = 75), or prostate (n = 36) cancers. Male to female ratio was 165/228, and median age was 60 years (range: 18-85). Acute (WHO and CTC 2.0 scales) and late (RTOG/EORTC scale) toxicities were graded in all patients. Any grade > 2 toxicity was considered as an event in terms of statistical evaluation. Six patients refused radiation therapy following blood collection and were, therefore, excluded from analyses. Median follow-up period for all treated patients (n = 393) was 29 months (range: 1-73).

Results: Following 8-Gy irradiation, median CD4 and CD8 apoptosis was 12.46% (range: -3.63-45.48) and 20.68% (range: 3.38-70.41), respectively. No correlation was found between early toxicity and T-lymphocyte apoptosis. However, among the 393 patients, grade 2 and 3 late toxicity was observed in 94 (24%) and 25 (6.4%) patients, respectively. Radiation-induced CD4 and CD8 T-lymphocyte apoptosis significantly predicted grade 2 and 3 late effects. Among the patients who showed CD4 radiation-induced apoptosis below the median (n = 197), 92 presented grade > 2 toxicity compared to 27 above the median (n = 196; $p < 0.0001$). The same relation was observed for CD8 apoptosis as well: 105 grade > 2 late effects out of 197 *vs.* 14 out of 196 ($p < 0.0001$). Considering only grade 3 late toxicity, patients with late effects (25 out of 197) all showed CD4 or CD8 radiation-induced apoptosis below the median ($p < 0.0001$ for both CD4 and CD8). When analyzing major grade 3 toxicities separately, we observed significant relations between radiation-induced lymphocyte apoptosis and skin ($p < 0.0001$ for both CD4 and CD8), subcutaneous ($p < 0.0001$ for both), salivary glands (p = 0.0009 and < 0.0001 for CD4 and CD8, respectively), bladder (p = NS for CD4 but 0.02 for CD8), and intestinal (p = NS for CD4 but 0.002 for CD8) late toxicities.

Conclusion: To our knowledge, this is the first rapid assay of intrinsic radiosensitivity confirmed prospectively. It can predict significantly the differences in radiation-induced late toxicity between individuals, and could be used as a rapid screen for genetically hypersensitive patients. Patients in future dose escalation studies could be stratified using the apoptosis assay.

252 THE ROLE OF BIOLOGICAL MARKERS IN PREDICTING HYPOPHARYNGEAL CANCERS LIKELY TO RESPOND TO INTENSIVE CHEMO-RADIATION THERAPY

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Objective: A retrospective study of biopsies accrued during a previous randomised phase III clinical trial conducted between 1990 and 1995 is in progress. The trial compared immediate surgery followed by radiotherapy with induction chemotherapy followed by endoscopy, further chemotherapy and radiotherapy for responders. Results showed that laryngeal organ preservation by chemo-radiation can be achieved. The objective of the present study is to identify biological markers that may predict patients likely to respond to the chemo-radiation protocol.

Materials and methods: The trial recruited 202 patients, with 100 entered into the chemo-radiotherapy arm. Paraffin blocks of the initial hypopharyngeal biopsy material from the chemo-radiotherapy arm were obtained and immunohistochemistry performed to detect nuclear staining of p53, p16, p21 and pRb proteins. In order to assess the role of chronic hypoxia, immunohistochemistry was also performed for GLUT-1 and CD31/34. A metaPCR has been developed and validated for rapid mutation analysis of exons 6/7/8, 4a9 and 4a5 of the p53 gene.

Results: The immunohistochemical results show a positive correlation between p21 and p53 expression and a weak inverse correlation between p53 staining and pRb. There was positive correlation between GLUT-1 expression and p53 staining. Loss of p16 expression was seen in 53% of cases. Mutation analysis for alterations in p53 'hotspots' showed that immunohistochemistry lacked sensitivity for use as a biomarker.

Conclusions: No predictive pattern of biomarkers has yet emerged and the sample size will have to be increased to permit a multivariate analysis. Mutation analysis is needed as a gold standard for analysis of the p53 tumour suppressor gene in hypopharygeal cancers and the metaPCR has proven to be a reliable and rapid method for such analysis. The p16 gene is altered at least as frequently as the p53 gene in hypopharyngeal cancer.

253 THE USE OF TISSUE MICRO ARRAYS IN PREDICTING THE OUTCOME OF PATIENTS WITH RECTAL CANCER

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Purpose: Our aim is to predict the variable response to preoperative chemo-radiation for rectal cancer. For this purpose, we have set up the tissue microarray technique (TMA) to study the expression of endogenous markers of proliferation (Ki67) and hypoxia (CA-IX), each thought to affect the outcome of radiotherapy and/or surgery. TMA enables the study of large numbers of patients concurrently, to perform uniform stainings, to reduce the cost of antibodies and to reduce manual interaction. Here we present our preliminary findings with this technique.

Material and methods: Between 1995 and 2000, 58 patients with a T3-4anyNM0 adenocarcinoma of the rectum were neoadjuvantly treated with radiotherapy alone (30 Gy in 10 fractions) or with a combination of radiation (45 Gy in 25 fractions) and chemotherapy (5FU-leucovorin as a bolus during the first and the last 5 days of the radiation). TMAs were constructed using a tissue-arraying instrument consisting of thin-walled stainless steel biopsy needles and stylets used to empty and transfer the needle content. The cylindrical core samples from paraffin-embedded tissue specimens were arrayed at high density into a recipient TMA paraffin block. Morphologically defined regions of the tissue blocks were identified using a hematoxylin and eosin (H&E) stained section from each donor block. Each TMA was constructed according to a fixed pattern. Tissue from the initial biopsy was punched (3 cores/patient), followed by 4 to 6 core biopsies from different areas from the surgical specimen, then 3 cores containing normal mucosa, and finally 2 cores from positive lymph nodes. Each fifth slice was stained with H&E to check the representativity of the core biopsies. Up to 40 consecutive5μ sections were cut from every TMA block. From each block, one section was stained for Ki-67 and one for CA-IX. To assess proliferative activity and hypoxia, serial sections were stained with primary antibodies against Ki-67 and CA-IX, followed by standard immunoperoxiase/DAB detection. A Ki-67