Purpose or Objective: We aimed to evaluate the pathological hypoxic and perfusion effects of Trastuzumab (T) and/or Cisplatin (C) in HER2+ oesophageal adenocarcinoma xenograft (OE19) which may potentially direct future clinical adjunctive therapy.

Material and Methods: SCID mice (n=17) bearing subcutaneous OE19 tumours were treated with either (i) Cisplatin 4mg/kg once a week, (ii) Trastuzumab 20mg/kg twice a week or (iii) Cisplatin and Trastuzumab for 2 weeks. Intraperitoneal Pimonidazole (Pm), an exogenous hypoxic marker, and intravenous Hoechst 33342 (Ho), a perfusion marker, were injected 2 hours and 1 minute prior to tumour excision, respectively. Tumours were immediately snap-frozen and 10µm frozen sections were obtained for immunofluorescence study. Following fixation, non-specific binding was blocked using 10% normal goat serum. The sections were then incubated overnight at 4°C with primary Pimonidazole FITC labelled mouse monoclonal antibody to 1:25 concentration. Propidium iodide (PI) was used as a counterstain to highlight morphology. Tumour sections were scanned using different filters for Pm (green), Ho (blue) and PI (red) on a fluorescence microscope at x100 magnification (Figure 1).

Results: Overall, tumour periphery was better perfused in most tumours but there was no consistent hypoxic intratumoral spatial localisation. There was an inverse spatial relationship between Pm and Ho fluorescence in 10/17 tumours, colocalisation in 3/17 and no relationship found in 4 tumours. Trastuzumab-treated tumours (HF 38%±17) were less hypoxic compared to the NT group (HF 50%±13) and these tumours were also better perfused (PF: T 46%±25, NT 39%±16). Cisplatin-treated tumours had the highest HF (50%±13) and lowest PF (39%±16) compared to Trastuzumab (HF 34%±13, PF 48%±26) and combination therapy (HF 41%±21, PF 45%±27).

Conclusion: Trastuzumab appeared to exert the predominant proangiogenic effect with improved perfusion and reduced intratumoral hypoxia, although these effects were diminished with combination therapy. These data suggest that the addition of hypoxia-modifying agents might be tested as an adjunctive therapy, particularly in those not eligible or fit for Trastuzumab therapy.

Poster: Radiobiology track: Normal tissue effects: pathogenesis and treatment

Impact of Ramipril on rat spinal cord after high- and low-LET irradiation
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Image analysis was performed using the ImageJ software. Percentage areas stained with Pm (hypoxic fraction/HF) and Ho (perfusion fraction/PF) were derived and mean (%) ± SD are presented. Difference in the HF and PF between Trastuzumab (T) and non-Trastuzumab (NT) treated animals were analysed.

Conclusion:

Trastuzumab appeared to exert the predominant proangiogenic effect with improved perfusion and reduced intratumoral hypoxia, although these effects were diminished with combination therapy. These data suggest that the addition of hypoxia-modifying agents might be tested as an adjunctive therapy, particularly in those not eligible or fit for Trastuzumab therapy.
Purpose or Objective: In radiotherapy of head and neck cancer the central nervous system is the dose limiting factor. Late side effects may occur which severely impair the patient’s quality of life. Thus, to improve the therapeutic ratio, radioprotective drugs receive increasing interest. In the optimal case, they could protect the normal central nervous system without influencing the tumor response to irradiation. A lot of studies using various approaches with e.g. melatonin, pentoxifylline, growth factors, Amifostine or Angiotensin converting-enzymes inhibitors (ACEI) were performed focusing on mitigation or, ideally, on protection from late side effect in central nervous system (brain, optic nerve or spinal cord).

Material and Methods: Within our study the impact of ACEi Ramipril on prevention from the late side effect radiation-induced myelopathy (forelimb paresis grade II) was tested. The cervical spinal cord of female Sprague Dawley rats was irradiated with either 6 MeV photons or carbon ions (12C-ion) (a linear energy transfer (LET) of 45 keV/µm and a 6 cm spread-out Bragg Peak was used). Immediately after irradiation (RT) Ramipril (2 mg/kg/day) was given via the drinking water for 300 days. A total of four groups were used: (1) photon RT + Ramipril (n = 24), (2) photon RT only (n = 20), (3) 12C-ion RT + Ramipril (n = 20) and (4) 12C-ion RT only (n = 20). For each group a complete dose-response curve after single dose irradiation was established and TD50-values (dose at 50% complication probability) were determined for the development of paresis grade II within 300 days.

Results: Preliminary analysis of the data shows no marked shift of the TD50-values related to administration of Ramipril after 12C-ion or photon RT, however, a prolongation of latency time for both irradiation modalities was found. At a dose level of 21 Gy the minimum latency time after 12C-ion RT was 160 d compared to 191 d after 12C-ion RT + Ramipril administration. Whereas, at a dose level of 26 Gy the minimum latency time after photon RT was 225 d after photon RT + Ramipril administration. Overall the latency time after 12C-ion RT was shorter compared to photon RT.

Conclusion: Ramipril administration after 12C-ion or photon RT exhibits a prolonged latency time. However, to find an ideal radiomitigator further examinations of the underlying pathological mechanisms leading to radiation-induced myelopathy are necessary. Additionally, since it is unclear how Ramipril interferes the pathological mechanism(s) of radiation-induced damage, it is important to understand the underlying mechanism. Thereby it would be possible to compensate potential weak points in inhibition by combination with other compounds.

PO-0991
p53 and in vitro radiation response of fibroblasts from RT-sensitive and -resistant patients
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Purpose or Objective: To test the association between the molecular and functional radiation response of fibroblasts in vitro and breast cancer patients’ risk of late reaction after radiotherapy.

Material and Methods: Fibroblast cultures were established by outgrowth from biopsies taken with informed consent from selected breast cancer patients with minimal (RT-resistant, n=15) or marked breast changes (RT-sensitive, n=19) after breast conserving therapy. The clinical risk of RT-sensitive patients was further ranked according to severity relative to external risk factors. Experiments were performed in vitro with 4 Gy or sham irradiated. Molecular markers p53, p21/CDKN1A, p16/CDKN2A, α-sma, and Ki-67, were detected by immunofluorescence microscopy at 2h, 2 and 6 days after irradiation (IR). Plating efficiency (PE) and surviving fraction after 4 Gy (SF4) were determined by the colony formation assay. Non-parametric analysis of differences between fibroblasts from RT-sensitive and RT-resistant patients was performed with the Wilcoxon/Mann-Whitney test, and correlations using the Spearman’s ρ rank correlation test.

Results: The basal level of p53 without irradiation was significantly higher in fibroblast cultures from RT-sensitive relative to RT-resistant patients (P=0.02). p53 was upregulated 2h - 2 days after IR in all cells but decayed more slowly on day 6 in fibroblasts from RT-sensitive patients. Further, explorative analysis showed strong early upregulation of p53 2h after irradiation in fibroblasts from high-risk patients (P=0.002). RT sensitivity showed no significant correlation with p21/CDKN1A, p16/CDKN2A, α-sma, and Ki-67, or functional endpoints, PE and SF4. However, proliferation activity (Ki-67 index) appeared to have a confounding influence on the effect of p53. Differential expression of p53 was correlated with basal levels of p53 (P<0.001) in unirradiated cultures with lower Ki-67 whereas it correlated with early upregulation at 2h (P<0.001) in cultures with higher Ki-67. Furthermore, correlations of p21/CDKN1A with p53 or p16/CDKN2A were markedly different in fibroblasts from RT sensitive and RT-resistant patients.

Conclusion: In this cohort, patient selection was performed to enhance the contrast between RT-resistant and RT-sensitive patients, including rare patients with severe late reaction. p53 levels in fibroblast cultures in vitro were significantly correlated with the risk of developing late breast changes after radiotherapy, and high-risk patients’ fibroblasts showed strong early upregulation of p53 after irradiation which depended on the proliferation index. We suggest that a relation between p53 and the risk of late reaction exists in a subgroup of RT-sensitive patients, possibly via enhanced genetic instability and partial dysregulation of the DNA damage response.

PO-0992
The role of HIF-1 in the neo-vascularization of the rectal mucosa after radiation therapy.
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Purpose or Objective: Rectal bleeding after radiation therapy (RT) for prostate cancer has been observed in up to 40% of patients and it is mainly due to multiple rectal angiectasias developed after RT. Soon after the beginning of RT, there is an acute mucosal reaction that can evolve into a more severe condition with prominent vascular involvement, evidence of vasculitis, arteriole thrombosis and subsequent ischemia and angiogenesis. Recently, attention to the role of hypoxia has contributed to the understanding of radiation-induced late normal tissue response. Under hypoxic conditions, the diverse hypoxia-driven genes (e.g., VEGF) are regulated by a transcriptional factor, hypoxia-inducible factor-1 (HIF-1). In vivo and in vitro studies have shown that the HIF-1 expression increased soon after irradiation, reaching the highest level after 30 days and preceding the expression of VEGF.