Purpose or Objective: 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. Early and late fibroblasts were irradiated 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 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 after 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. Furthermore, basal levels of p53 were correlated with Ki67 and correlated with early upregulation at 2h (P<0.001) 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 fibroblasts 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, arteriolar 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.
Aim of this prospective study is to evaluate the expression of HIF-1 after RT and correlate it with the development of rectal mucosal angiostasias and bleeding.

Material and Methods: Patients with histological proof of prostate cancer without distant metastases, undergoing a standard course of external beam radiation therapy (3D-RT), were considered eligible. Each patient underwent a rectosigmoidoscopy with bioplastic sampling prior to and one month and one year after RT. The development of rectal mucosal angiostasias was graded according to the Vienna Rectoscopy Score (VRS). HIF-1 was evaluated by immunohistochemistry and western blot analysis; the mean number of blood vessels per field was also assessed. Radiation-induced side effects (e.g. rectal bleeding) were recorded during follow-up visits.

Results: Thirty-one patients were enrolled (median age 72 years, IQR 67-75). After the end of a median follow-up of 19.8 months (IQR 18.4-20.9), 10 patients (32.3%) developed rectal bleeding needing intervention. All these patients presented a grade II or III VRS (p=0.03). The difference in the mean number of blood vessels between bleeders and nonbleeders was not significantly different (p=0.47). The expression of HIF1 in bleeding patients was down regulated in 2 cases, unchanged in 3 and up regulated in 4 cases (p=0.99); in one case it was not possible to determine the expression. There was no correlation between the expression of HIF1 and the VRS.

Conclusion: The expression of HIF1 does not correlate with the development of rectal mucosal angiostasias and bleeding in patients irradiated for prostate cancer.

PO-0993
Genetic profiles of glioblastoma in proximity to the subventricular zone receiving chemoradiation
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Purpose or Objective: Subventricular zone-infiltrating (SVZ-infiltrating) glioblastomas (GBMs) with subependymal spreads along ventricle walls are associated with decreased patient survival. The heterogeneity in patient survival and recurrence patterns of GBM with SVZ infiltration might be related to neuronal therapy-resistant tumor cells, located in the SVZ. It has not been systematically investigated if specific molecular genetic patterns of SVZ-infiltrating GBMs exist, and therefore are responsible for the unfavorable course after chemoradiation.

Material and Methods: The current study assessed the molecularbiologic profile of 55 primary GBM cases that underwent chemoradiation. GBMs with SVZ infiltration and subependymal tumor spread (n = 24; 43.6 %) and peripherally located GBMs (n = 31; 56.4 %) were included. Genome methylation patterns were determined and copy number profiling was performed using an Illumina Infinium HumanMethylation450K (450K) Array, and the prognostic influence on progression and survival was evaluated.

Results: The majority of patients showed the characteristics of a “classic” GBM subtype, independent of the tumor localization in regard of the SVZ, demonstrating a chromosome 7 gain and chromosome 10 loss, as well as deletion of Cyclin-Dependent Kinase Inhibitor 2A (CDKN2A) and amplification of Epidermal Growth Factor Receptor (EGFR). Second, RTK I subtype, showing Platelet-Derived Growth Factor Receptor Alpha (PDGFRA) amplifications, could be detected equally in both groups. However, SVZ-infiltrating GBMs with subependymal spreading showed a decreased overall survival (OS) compared to their peripheral counterparts.

PO-0994
Assessment of [11C]-metformin PET for identification of patients suitable for metformin treatment
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Purpose or Objective: Evidence to support a role for the antidiabetic drug metformin in the prevention and treatment of cancer has emerged over the last decade. In particular, recent studies demonstrate that metformin enhances tumor response to radiation in experimental models. Metformin may therefore be of utility for nondiabetic cancer patients treated with radiation therapy. Despite being in clinical use for almost 60 years, the underlying mechanisms for metformins action remain elusive. We have therefore applied a novel PET-tracer, [11C]-metformin, to determine the uptake mechanism and elimination of the drug in vitro and in vivo.

Material and Methods: To verify transporter-mediated uptake of metformin in tumor cells, a selection of cell lines were incubated with [11C]-metformin in the absence or presence of blocking unlabelled metformin. Two tumor models A549 (lung) and SiHa (cervix) was chosen for in vivo experiments. Mice bearing subcutaneous tumors in the lower back were administered ~10 MBq [11C]-metformin and dynamically PET scanned for 90 minutes. As a “proof of principle” experiments using PET/CT with [11C]-metformin organ specific uptake of [11C]-metformin was determined in healthy humans. Dynamic whole-body PET was performed on four healthy volunteers (2 male). Two minutes before scan start, a bolus injection of ~200 MBq [11C]-metformin was injected and five consecutive whole-body scans with increasing frame durations were obtained: 1, 1.5, 2, 2.5 and 3 minutes per bed position. Time intervals for the PET scans were 2-8, 9-18, 19-32, 33-48 and 49-67 minutes (see figure 1). Source organs for the dosimetry calculations were the liver, kidneys, salivary glands and the bladder.

Results: In vitro metformin uptake varied widely but a high and inhibitable uptake was observed in A549 and SiHa cells.