Aim of this prospective study is to evaluate the expression of HIF-1 after RT and correlate it with the development of rectal mucosal angiectasias 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 angiectasias 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 non bleeders 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 feasible 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 angiectasias and bleeding in patients irradiated for prostate cancer.

**Poster:** Radiobiology track: Biomarkers and biological imaging

**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 venricular 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 resistance. Ependymal cells, located in the SVZ, have 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.

**Figure:** Genome wide copy number profiling of a classic primary glioblastoma with chromosome 7 gain and chromosome 10 loss

**Conclusion:** Genome methylation patterns were distributed independently of tumor localization in regard of the SVZ, suggesting that the biological entities in both GBM groups are identical. However, survival rates of GBMs with proximity to the SVZ were inferior and therefore the central localization seems to be responsible for the poor clinical courses.

**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 actions remain elusive. We have therefore applied a novel PET-tracer, [11C]-metformin, to determine the uptake mechanism and elimination of the drug in vivo and in vitro.

**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.
Imaging with [11C]-metformin in tumor bearing mice showed a large uptake in the kidneys and excretion through the bladder, as expected for metformin. An uptake of [11C]-metformin was seen in both A549 (lung) and SiHa (cervix) tumors and autoradiography supported this finding. Biodistribution of metformin in humans is shown in figure 1 with visible uptake in liver, kidney and the salivary glands, but no detectable uptake in brain, muscle or adipose tissue.

Conclusion: It is possible to visualize distribution of [11C]-metformin in vivo. In xenograft models uptake in tumor was seen. It will be of great interest to investigate whether it is possible to visualize an uptake in human tumors, which will be done in a planned study in prostate cancer patients.

Poster: Radiobiology track: Cellular radiation response

PO-0995
Osteopontin expression in glioblastoma - a promoter of the cancer stem cell-like phenotype?
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Purpose or Objective: A high level of circulating osteopontin (OPN) at the end of radiotherapy (RT) is an adverse prognostic factor in patients with glioblastoma (GBM) and other tumours including rectum cancer. Recent mechanistic studies demonstrated HIF-2α-mediated OPN/CD44 promotion of the glioma stem cell-like phenotype in a mouse model. Using unique paired tumour samples from patients with GBM, we investigated changes in levels of OPN protein expression following RT and compared these with rectum cancers from patients irradiated with the same pre-operative fractionation.

Material and Methods: 3 patients with histologically confirmed GBM received pre-operative RT in an ethics-approved Phase I trial. 2.5 Gy b.d. was delivered using IMRT over 5 days. Maximal safe tumour resection was performed at 3, 5 and 10 days post RT in patients 1, 2 and 3 respectively. Immunohistochemistry was performed on the paired diagnostic biopsy and irradiated resection specimen using validated antibodies (rabbit polyclonal antibody to OPN: clone PA1-38332, Thermo Fisher Scientific) and an automated immunostainer. The staining was scored by a board-certified pathologist.

Results: Levels of OPN in GBM tumour cells were high at baseline as compared with rectum adenocarcinoma. There was marked increase in OPN expression in response to RT in all three GBM tumours (Fig 1). Expression of Glut-1, a marker of intrinsic hypoxia and a target of HIF-2α, was not induced. Ki67 levels were reduced although levels of cyclin D1 expression were unchanged. A dynamic contrast-enhanced (DCE) MRI performed on the last day of RT did not detect any change in tumour perfusion in any of the GBMs. Resection specimens from 3 rectum cancer patients irradiated preoperatively with the same schedule showed very low level induction of OPN.

Conclusion: RT increased the levels of OPN expression in GBM tumour cells. This may be a direct effect or related to RT-induced changes in the hypoxic tumour microenvironment that were not detectable on a DCE-MRI or by Glut-1 expression. Although RT significantly increases overall survival compared with surgery alone, particularly when combined with temozolomide, it may promote the cancer stem cell-like phenotype of residual GBM cells. Enhanced OPN/CD44 signaling in the perivascular niche is associated with resistance to therapy and blockade of this signalling pathway may prove of clinical benefit. The relative lack of induction of OPN expression in rectum cancer may explain the success of short course pre-operative RT in this tumour type.

PO-0996
Distinct radiation responses after mtDNA depletion are potentially related to oxidative stress
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Purpose or Objective: In process like reactive oxygen production and apoptosis mitochondria play an important role and both processes play also a significant role in radiotherapy (RT) response. Repair of RT induced damage is dependent on mitochondrial energy supply suggesting a role for mitochondrial DNA (mtDNA) in RT. mtDNA variations, such as mutations or depletion, might therefore influence RT response, as for example found in cisplatin-treated patients. Therefore carefully elucidating the effect of these processes in radiation response might be important. Hence, we hypothesize that reduced mitochondrial function enhances the radiation response as a consequence of reduced ATP production and increased cellular ROS exposure (Fig.1).