lifespan shortening and cancer risk of mice after irradiation in utero and treatment with N-ethyl-N-nitrosourea postnatally.

**Material and methods** Female B6C3F1 mice were either irradiated with 2 Gy X-rays at embryonic day 17 (X-ray alone) or administered with 125 ppm N-ethyl-N-nitrosourea (ENU) for 4 weeks from 5, 9, or 13 weeks old (ENU alone). Another group of mice were both exposed to X-rays in utero and administered with ENU postnatally, i.e., 5, 9, or 13 weeks old (X-rays+ENU). Control group were treated with sham-irradiation and vehicle-only. All mice were analysed for the life-span shortening and tumour spectrum histopathologically at moribund or just after death.

**Results and discussions** The mean lifespan of control mice was 797+/−143 days. In utero X-ray exposure shortened lifespan by 8.5%. The mean lifespan of mice treated with ENU alone at 5, 9 and 13 weeks of age were 366+/−117, 461+/−104, and 475+/−123 days, respectively. In utero exposure shortened lifespan of mice postnatally treated with ENU at 5, 9 and 13 weeks of age by 16.8, 9.0 and 7.5%, respectively, indicating that estimated risk of in utero exposure was enhanced by two-fold in mice treated with ENU at juvenile (5 weeks). This enhancement is in part ascribed to acceleration of tumour development such as thymic lymphoma. In contrast, the risk of lifespan shortening after in utero exposure was not influenced by ENU treatment when ENU was treated after adults (9 and 13 weeks). Histopathological examination is now undertaken in order to clarify the tumours whose risk is increased by in utero exposure in control and ENU treated mice.

**Conclusion** The risk of in utero exposure to X-rays was influenced by postnatal treatment with ENU, which depends on the age of ENU treatment. Increase in risk of in utero exposure by juvenile ENU treatment was ascribed to acceleration of tumours such as thymic lymphoma.

**PO-118 IMPACT OF PARITY ON NEUTRON-INDUCED MAMMARY CANCER RISK IN SPRAGUE-DAWLEY RATS**

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**Introduction** Breast cancer is one of the most common cancers in women throughout the world. It is well known that ionising radiation is a potent carcinogen of breast, and its effect is modified by both age at its exposure and the reproductive history. The atomic bomb survivor study suggests that radiation-related risk of breast cancer of women is influenced by the ages of first menarche, first birth and menopause. With respect to radiation carcinogenesis, quality of radiation, such as density of ionisation and energy of radiation, is a critical determinant of the risk of radiogenic cancer. Experimental studies have shown that densely-ionising neutrons exhibit an increased risk of cancer compared to sparsely-ionising gamma and X rays. Little data are available, however, about the effect of parity (i.e. history of pregnancy) on carcinogenesis induced by neutrons.

**Material and methods** Virgin female Sprague-Dawley rats at 35 weeks after birth (early middle age) were whole-body irradiated with gamma rays (Cs-137, 2 and 4 Gy) or fast neutrons of a mean energy of 2 MeV (0.05, 0.2 and 0.5 Gy). Parous rats, which gave birth and breast fed twice prior to 26 weeks of age, were also irradiated at 35 weeks. Rats were observed until 100 weeks and the incidence for mammary carcinoma, as compared to the non-irradiated group, was determined based on palpation records and pathological examination.

**Results and discussions** Susceptibility to radiation in induction of mammary cancer is clearly dependent upon age at exposure. Gamma-irradiation significantly induced mammary cancer in virgin rats at 15 weeks old (mature young adult) with a similar extent to that at 3 weeks (before puberty) in our previous study, whereas the susceptibility was lost at 35 weeks. Neutron exposure at 35 weeks, however, still increased the risk of mammary carcinoma, suggesting that the susceptible age window for breast cancer induction was wider for neutrons than gamma rays. Importantly, parity at the time of exposure completely inhibited an increase in mammary cancer risk irrespective of radiation type. Pathological examination to identify if parity influences a specific subtype of mammary carcinomas is now undertaken. The above evidence sheds light on the precise estimation/prediction of breast cancer risk of nulliparous and parous women, for instance, in case of diagnostic and therapeutic radiation exposure.

**Conclusion** This study suggests that parity significantly suppresses not only gamma-ray– but also neutron-induced mammary cancer.

**PO-119 ACCELERATED GLUTAMINE METABOLISM IS CONFLERING RADIORESISTANCE TO PROSTATE CANCER**

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**Introduction** Pathophysiological conditions within the tumour microenvironment induce metabolic adaptations of cancer cells. These metabolic features correlating with aggressive tumour growth pattern and the relapse risk after radiotherapy. Beside glucose, fast-growing cancer cells consume glutamine for energy production. Within the presented project we investigated the potential of the glutamine metabolism as putative therapeutic target and predictive biomarker for an individualised radiotherapy of prostate cancer (PC) patients.

**Material and methods** Genome and metabolome analysis of radioresistant PC cells identified the glutamine metabolism as regulator for intrinsic sensitivity to irradiation. Radiosensitizing effects of glutamine deprivation, molecular targeting and
genetic suppression of key enzymes were determined for clonogenic survival, DNA repair and sphere-formation ability to determine the cancer stem cell (CSC) potential in PC cell lines, primary culture models and ex vivo treated primary biopsies. Analysis of tumour growth after glutamine deprivation were performed in xenograft models. The intracellular level of the glutamine metabolism and tricarboxylic acid cycle metabolites, reactive oxygen species (ROS) and glutathione (GSH) were determined. The clinical validation of the identified metabolic biomarkers was carried out with the NanoString technology.

**Results and discussions** Radioreistant PC cells exhibit an accelerated glutaminolysis with enhanced α-ketoglutarate to succinate ratio. The elevated α-ketoglutarate is leading to GSH consumption, increased intracellular ROS level, modulated epigenetic regulators and induction of a CSC phenotype. Metabolic, chemical and genetic targeting of glutaminolysis results in the inhibition of mTOR signalling, enhanced endoplasmic reticulum stress and reduction of DNA repair. In combination with irradiation this targeting therapy is effectively radiosensitizing PC cells in vitro and in vivo. Moreover, the c-MYC gene expression, a key regulator of glutaminolysis, significantly correlates with the PSA-free survival after radiotherapy.

**Conclusion** This study shows that the enhanced glutaminolysis of PC cells is conferring resistance to radiotherapy. The therapeutic targeting of the glutamine metabolism is elevating the cytotoxic effects of irradiation. In addition, metabolic enzymes involved in the glutamine metabolism can be potentially used to predict clinical outcome of PC patients after radiotherapy.

**PO-120**

**METASTATIC BREAST CANCER: UNDERLYING MOLECULAR MECHANISMS OF RADIATION RESPONSE**

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**Introduction** Metastatic breast cancer (BCa) is an incurable disease with limited therapeutic response. Radiation therapy (RT) is widely used in the management of metastatic BCa, however, efficacy of conventional RT is often not sufficient due to occurrence of resistances. It is suspected that BCa cells with increased invasive potential exhibit intrinsic radiation resistance; however, there is a lack of models that allow investigating the underlying mechanisms. Therefore, the aim of this project is to establish BCa cell lines with increased invasive potential (INV), and to elucidate differences in radioresponsiveness compared to parental cell lines.

**Material and methods** MDA-MB-231, T47D and Au565 were subjected to repetitive migration through an uncoated 8 μm-pore membrane to obtain INV BCa cells. Invasive potential was validated by CytoSelect Invasion Assay (Collagen I or Laminin). Metastatic abilities of INV versus parental cells were determined in vivo using nude mice. Radiation response was evaluated by clonogenic assay. Deep Profiling Mass Spectrometry assays (MyOmix Ds, USA) was also performed.

**Results and discussions** While MDA-MB-231-INV cells invaded both collagen-I and laminin-coated membranes with a comparable efficiency, T47D-INV and Au565-INV cells showed pronounced invasiveness through Laminin. In in vivo experiments tumours formed by INV cells grew slower at the injection site but, importantly, lead to increased lymph node size, thus confirming increased metastatic potential. After exposure to photon-based ionising radiation MDA-MB-231 parental and INV cells exhibited comparable clonogenic survival. However, while T47D-INV cells display increased clonogenicity, Au565-INV cells revealed lower clonogenic capacities after irradiation, compared to their parental counterparts. Moreover, we identified a number of proteins that were differentially regulated in INV compared to parental BCa cells, demonstrating a close relationship between invasive and migratory capacities and cell death, cell cycle regulation and DNA damage response.

**Conclusion** We successfully established 3 BCa cell models with increased invasive capacities. Our results demonstrate that enhancement of invasiveness is accompanied by modulation of cell migration, resulting in increased metastatic potential of BCa cells in vivo. The differences in the sensitivity to irradiation in the 3 INV cell lines are pointing to a heterogeneous regulation of radiation response in different BCa subtypes.

**PO-121**

**LENTIVIRALLY-DELIVERED SHRNA KNOCKDOWN OF CXCL12 PREVENTS FIBROSIS IN A RODENT MODEL OF RADIATION LATE ADVERSE EFFECTS**

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**Introduction** Late Adverse Effects (LAEs) following adjuvant radiotherapy (RT) are common sequelae in free flaps used for breast reconstruction. Intra-arterial delivery of gene therapy to an isolated vascular region permits localised, targeted genetic modulation of reconstructed tissue, avoiding potential off-target effects in microscopic residual disease. We hypothesised that targeted knockdown of CXCL12 would reduce LAEs.

**Material and methods** LAE development was studied across 8 time points to 90 days after 50 Gy/3 in 24 male Fischer 344 rats in a validated LAE flap model. RT and control human skin and subcuticular tissue was obtained for validation from the PRADA study.

Efficacy of ShCXCL12 against LAEs was assessed in 72 animals. 1 × 10^9 lentiviral particles encoding ShRNA against CXCL12 (ShCXCL12), RNA overexpressing CXCL12 (OeCXCL12), or scrambled control (SCR), or sham infection with PBS were delivered intra-arterially to the flap tissue 30 minutes before RT. Acute and Late toxicities were assessed using Radiation Therapy Oncology Group (RTOG) scores with tissue endpoints at 7, 21 and 90 days after RT.

**Results and discussions** CXCL12 and CXCR4 were up-regulated after RT, peaking at 7 days (p=0.001 and p=0.047 respectively). Confocal imaging co-localised CXCL12 and γH2AX in fibroblast stromal cells after RT, confirming that CXCL12 was flap-originating and suitable for our therapy. This was associated with increased macrophage ingress and fibroblast activation characterised by immunohistochemical and flow cytometric end-points (p=0.035). We corroborated these findings in human RT tissue, demonstrating increased CXCL12 (p=0.035) and CD68 (p=0.027) expression compared to paired controls.

ShCXCL12 animals had reduced acute toxicities compared to LVSCR or PBS groups. OeCXCL12 animals had