Fourth International Conference on Precision Image-Guided Small Animal Radiotherapy Research

12-14 March 2018
Lisbon, Portugal

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1. Introduction to the 4th International Conference on Precision Image-Guided Small Animal Radiotherapy Research

The organizers of the 4th International Conference on Small Animal Radiotherapy Research would like to welcome you to Lisbon. This initiative grew from the realization that the new research field of precision image-guided small animal radiotherapy needs to bring together many disciplines to lead the field to fruition. A first very successful Symposium was held in Maastricht (Netherlands) in March 2013, followed by the second one in Vancouver in 2014, and a third one in Ghent, Belgium, in 2016.

The technological breakthroughs in animal imaging and precision irradiation have enabled experimental studies with research questions with a complexity which until recently could not be addressed. We can now, with unprecedented accuracy, proceed to discover radiation interaction mechanisms in whole organisms. New tumor models are emerging all the time. There is much hope to discover new synergistic effects of e.g. drugs or other agents, combined with radiotherapy. This new knowledge will help us in translating our findings towards human trials for combating several diseases, in particular cancer.

We brought together many of the leading specialists and vendors in the fields of radiobiology, radiotherapy, radiation physics, precision engineering, imaging, dose calculation, and others. We hope for a highly interactive two and a half day symposium with cutting edge science, stimulating discussions and debates, and we hope you may emerge from it with new ideas, new collaborations and an expanded research network.

Besides enjoying the Conference, we hope that you will also have time to enjoy the beautiful historic city of Lisbon.

We wish you an exciting Conference!

Prof Pedro Vaz, Dr Ana Belchior
Centro de Ciências e Tecnologias Nucleares
Instituto Superior Técnico
Universidade de Lisboa, Portugal

Prof Frank Verhaegen
Maastro Clinic
Maastricht, the Netherlands

2. Symposium Organization

Scientific Organizers
Prof Pedro Vaz, Dr Ana Belchior
Centro de Ciências e Tecnologias Nucleares –Instituto Superior Técnico
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Accessibility
Please consult the following website for all travel information to the Symposium venue:

http://small-animal-rt-conference.com/venue/

Internet access will be available throughout the conference:
Login data will be provided locally

3. Sponsoring and endorsements

We would like to gratefully thank our generous sponsors:

GOLD: Precision X-ray Inc

SILVER: Xstrahl Life sciences, Advacam, RaySearch, Perkin Elmer

BRONZE: Faxitron

The following organization is endorsing the Symposium: The European Society for Radiotherapy and Oncology (ESTRO)

4. Themed publication of Symposium papers in Br J Radiol

Br J Radiol is planning a **special feature on *Small Animal IGRT***, provisionally scheduled for early 2019, with *Guest Editors* ***Prof Pedro Vaz, Prof Frank Verhaegen*** and ***Prof Kevin Prise*** (BJR Editor-in-Chief (scientific)). The deadline for submitting papers for this issue is May 21 2018.

Instructions for authors can be found here:
http://www.birpublications.org/page/ifa/bjr

More information may be obtained from: Tami.Potten@bir.org.uk

5. Symposium program

Keynote Speaker

We're proud to open the Conference with a seminar by one of the leaders of radiobiology research, Professor Brian Marples from the University of Miami. Professor Marples will give his seminar "The molecular regulation of radiation nephropathy" on Monday March 12 in the first session.

Invited speakers

Rebecca Bütof (University Clinic Carl Gustav Carus, Technical University Dresden, Germany)
Chris Vanhove (Ghent University, Belgium)
Juergen Meyer (University Washington, Seattle, USA)
Susana Constantino (Cardiovascular Center, University of Lisbon, Portugal)
Peter van Luijk (University Medical Center Groningen, the Netherlands)

Poster session
Throughout the Symposium all posters will be on display. There are no dedicated poster viewing times. Vendors will exhibit in spaces near to the conference room.

Monday March 12

8.00-9.00	Registration
9.00-9.10	Opening address: P Vaz, A Belchior, F Verhaegen (Lisbon, Maastricht)
9.10-9.55	**KEYNOTE ADDRESS (Chair: F Verhaegen)** B Marples - The molecular regulation of radiation nephropathy
	Setting up radiation studies. Chair: F Verhaegen
9.55-10.25	INVITED: P van Luijk - Rationale and study design for in vivo studies on normal tissue damage using high-precision irradiation techniques
10.25-10.40	R Endersby - Replicating clinical radiation therapy protocols in preclinical brain tumour models
10.40-11.10	**Coffee break**
	Tumor targeting. Chair: P van Luijk
11.10-11.40	INVITED: R Bütof - Establishment of orthotopic lung and brain tumors for image-guided radiation therapy with photons and protons
11.40-11.55	K Butterworth - Inhibition of ataxia telangiectasia related-3 (ATR) improves therapeutic index in preclinical models of non-small cell lung cancer (NSCLC) radiotherapy
11.55-12.10	I Grgic - Tumor reoxygenation and image-guided SBRT for the treatment of murine colorectal liver metastases
12.10-12.25	T Hellevik - Optimizing Radiotherapy-Immunotherapy combinations in preclinical models
12.25-12.45	Discussion Session I- Moderators: A Sawant, P van Luijk
12.45-14.00	**Lunch**
	Normal tissue targeting. Chair: R Bütof
14.00-14.15	R Hill - Preclinical Assessment of Late Gastrointestinal Toxicity with the Addition of Plerixafor to Radiochemotherapy for Cervical Cancer
14.15-14.30	N Melin - Radiation induced liver disease mouse model; a promising step toward understanding of normal liver tissue toxicity
14.30-14.45	L Schyns - Murine versus human tissue compositions: implications of using human tissue compositions for dose calculations in mice
14.45-15.00	M Ghita - Preclinical Characterisation of Radiation Dose-Volume Effects on Early and Late Lung Toxicity
15.00-15.15	J Hannan - Temporally impaired regenerative capacity and increased inflammation and apoptosis in pelvic neurons post-radiation therapy
15.15-15.45	**Coffee break**
	News from the manufacturers. Chair: C Vanhove
15.45-16.00	P Dejean (PXi)
16.00-16.15	A Treverton (XStrahl)
16.15-16.30	E d'Agostino (DoseVue)
16.30-16.45	R Nilsson (RaySearch)
16.45-17.00	E Trojanova (Advacam)
17.00-17.15	O Kelada (Perkin Elmer)
17.15-17.30	P Granton (SmART Scientific Solutions)
17.30-17.50	Discussion Session II - Moderators: R Bütof, C Vanhove
	Free evening

Tuesday March 13

Tumor targeting. Chair: B Marples	
9.00-9.30	INVITED – S Constantino - Low doses of ionizing radiation induce angiogenesis: benefits, concerns and challenges to health and science
9.30-9.45	A Sawant - Mild hyperthermia as a localized radiosensitizer for deep-seated tumors – Investigation in an orthotopic prostate cancer model in mice
9.45-10.00	M Dolera - Definitive high-dose hypo-fractionated stereotactic brain-sparing irradiation of stage IV canine nasal tumors: a feasibility study and first clinical experiences
10.00-10.15	S Kampfer - What safety margin for PTV creation should be used in preclinical radiotherapy of mice? A first approximation with a well-known margin recipe.
10.15-10.30	A Vaniqui - On the determination of planning target margins due to motion for mice lung tumours using a 4D MOBY phantom
10.30-10.45	S van Hoof - Evofosfamide sensitizes esophageal carcinomas to radiation without increasing normal tissue toxicity
10.45-11.15	**Coffee break**
Imaging & Novel methods. Chair: P Almeida	
11.15-11.45	INVITED: C Vanhove - Magnetic resonance based small animal radiotherapy in neuro-oncology
11.45-12.00	B Markelc - Combining intravital multiphoton microscopy and image guided radiotherapy to determine the response of tumour vasculature to irradiation
12.00-12.15	M Costa - The potential of photoacoustic imaging as a predictive and monitoring tool in radiotherapy of head and neck tumours
12.15-12.30	F Lallemand - Evaluation of tumor perfusion with IVIM analysis after radiotherapy
12.30-12.50	Discussion Session III – Moderators: B Marples, P Almeida
12.50-14.00	**Lunch**
Dosimetry & Technology. Chair: A Belchior	
14.00-14.15	A Subiel - Accurate and traceable dosimetry for pre-clinical radiobiological studies
14.15-14.30	B Yalvac - Response of alanine dosimeters to low energy X-rays and small fields
14.30-14.45	A Anvari - Investigating the use of the portal imager as a quality assurance tool for the small animal radiation research platform (SARRP)
14.45-15.00	Y Poirier - Dosimetric consequences of the use of thin filters in modern image-guided small animal irradiators
15.00-15.15	A Hunger - New approaches for studying radiobiological effects of kilovoltage X-rays in vivo and in vitro
15.15-15.30	K Sheng - Small Animal IMRT using Double-Focused Sparse Orthogonal Collimators
15.30-16.00	**Coffee break**
Proton beam studies. Chair: P Vaz	
16.00-16.30	INVITED: J Meyer - Spatially-modulated proton minibeams on the UW radiation platform for preclinical in vivo research
16.30-16.45	E Beyreuther - Establishment of small animal irradiation at University Proton Therapy Dresden
16.45-17.00	I Almeida - Exploring the capabilities of a clinical proton beam with a dynamic collimator for pre-clinical research
17.00-17.15	E Diffenderfer - Design and commissioning of an image-guided small animal radiation platform for proton radiobiology research
17.15-17.35	Discussion Session IV - Moderators: A Belchior, P Vaz
19.00	**Symposium Dinner (Restaurant Museu da Cerveja)**

Wednesday March 14

	Imaging & Novel methods. Chair: A Paulo
9.00-9.15	L Gano - Multifunctional agent for breast cancer radiotheranostics
9.15-9.30	S van Hoof - A novel preclinical data management platform to improve translational research
9.30-9.45	S Dobiash - Use of the liquid fiducial marker BioXmark for high-precision radiotherapy of an orthotopic pancreatic tumor mouse model
9.45-10.00	R Burkhardt - Optimization of X-Ray Dark-field microCT for Murine Imaging Studies of Radiation-Induced Lung Fibrosis
10.00–10.15	F Silva - Multifunctional Bioconjugated Gold Nanoparticles for Cancer Theranostics
10.15-10.45	**Coffee break**
	Dosimetry & Technology. Chair: J Meyer
10.45-11.00	F Tillner - *μ-RayStation 5*: Expanding functionality of a clinical treatment planning system towards application for image-guided small animal radiotherapy
11.00-11.15	M Walb - Quantifying the setup uncertainty of a stereotactic murine system using the image guidance of the X-RAD SmART irradiator
11.15-11.30	M Reinhart - μIMRT on the SARRP using the motorized variable collimator
11.30-11.45	F Verhaegen - ESTRO ACROP: Technology for precision small animal radiotherapy research: Optimal use and challenges
11.45-12.05	Discussion session V – Moderator: A Paulo, J Meyer
12.05-12.20	P Vaz, A Belchior, F Verhaegen – Best Young Speaker Awards & closing the Symposium

Posters

1. Lallemand	Brain stereotactic radiotherapy damages recorded by Functional MRI
2. Stevenson	Initial Treatment Plan and Delivery Accuracy Assessment of the Small Animal Radiotherapy Research Platform
3. Ye	Complete Response and slight side effect after IMRT for a feline unknown nasal tumor case
4. Khmelinskii	Longitudinal and regional assessment of radiation-induced lung density changes using automated image registration in μCBCT mouse data
5. van Hoof	Dose painting by combined couch motion and irradiation on an image guided small animal radiotherapy platform

Abstracts
Oral presentations

Radiation-induced kidney injury: Development of a murine model and mitigation of functional injury

B. Marples (1); A. Ahmad (1); A. Mitrofanova (2); Y. Yang (1); Y. Zeidan (1,3), A. Fornoni (2)

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Introduction: Radiation nephropathy (RN) occurs after radiotherapy. RN is functionally characterized by an initial acute phase (proteinuria) followed by a chronic decline of glomerular filtration rate leading to renal failure. RN is irreversible and affects the quality of life of both short- and long-term cancer survivors. The molecular mechanisms responsible for the development of proteinuria and glomerulosclerosis in RN remain largely unknown. The current study investigates the role of sphingolipids in radiation-induced podocytopathy.

Materials&Methods: Sphingomyelin phosphodiesterase acid-like 3B (SMPDL3b) expression was assessed in cultured podocytes after 8 Gy X-irradiation by real-time PCR (RT-PCR) and Western blotting. Morphological changes and DNA damage in irradiated podocytes were assessed using immunofluorescence microscopy. Wild-type C57BL/6 male and female mice (age 10–14 weeks) were irradiated using image-guidance (single dose 14 Gy and fractionated 20x2 Gy). In the mitigation experimental arms, 50 mg/kg Rituximab (or IgG) was administered IP 30 min before irradiation. Functional kidney parameters (proteinuria and albumin/creatinine urine ratio, along with serum BUN [blood urea nitrogen], creatinine and hematocrit), kidney histology, and gene expression were analyzed at 10- 40 weeks.

Results: Following irradiation, SMPDL3b expression at the protein level was significantly reduced *in vitro* and *in vivo*. However, no significant changes were observed at the transcriptional level. Podocyte number also decreased significantly post radiation *in vivo*. H&E kidney sections showed a multifocal increase in the number of pericytes, tubular atrophy, and glomerular damage. Periodic Acid-Schiff (PAS) staining showed an increase in glomerular mesangial matrix accumulation post-irradiation, along with diffuse intertubular fibrosis assessed by Sirius red staining, especially in the renal cortex. Rituximab improved kidney functional parameters, vascular structure, normalization of pericyte coverage, and suppressed the development of fibrosis and tubular damage post irradiation.

Conclusion: SMPDL3b expression was identified as a molecular determinant of podocyte injury after single dose and fractionated X-irradiation. Rituximab pretreatment protected mice against radiation-induced nephrotoxicity, which may have therapeutic implications for radiation-induced injuries in cancer patients.

Rationale and study design for in vivo studies on normal tissue damage using high-precision irradiation techniques

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Normal tissue damage plays a critical role in the optimization of radiotherapy. For some tumors the risk of normal tissue damage limits the dose that can be administered reducing the effectiveness of the treatment. In other patients the treatment leads to toxicity significantly affecting their quality of life.

Though normal tissue damage research can be performed clinically by assessing relationships between treatment parameters and toxicity, this approach is subject to limitations such as (but not limited to):

1) In clinical cohorts the number of parameters that vary is large. Though various epidemiological instruments are available to investigate associations between treatment parameters and toxicity, the particular characteristics of radiotherapy data pose severe limitations on the interpretation of such associations.

2) Clinical treatments are performed using strict guidelines. As a result important treatment parameters may not vary within clinical cohorts. Consequently, clinical data may provide limited information on mechanisms. Therefore optimizing treatment solely based on clinical data may miss opportunities to reduce toxicity.

3) Follow-up specifically informative for mechanisms is challenging due to due invasiveness, burden with associated low patient accrual, and cost.

These limitations provide a clear rationale for performing animal studies on normal tissue damage. Animal studies allow the design of controlled studies in which the investigator can choose which parameters to vary. Follow-up can be tailored to elucidate specific mechanisms. Elucidation of mechanisms will improve understanding of clinical observation, but can also inspire use of technology that would not be pursued based on clinical observations alone. In addition knowledge on mechanistic targets makes their targeting using other intervention strategies possible.

Though the aim is to achieve knowledge that can be translated to the clinic suggests that animal work should resemble clinical practice, the niche that animal studies occupy calls for distinct differences as well. To identify such differences, preclinical in vivo studies and methodology will be reviewed.

Replicating clinical radiation therapy protocols in preclinical brain tumour models

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Introduction: Brain cancers are the most common solid malignancies of childhood and second most common cancer diagnosis for people under the age of 15. These cancers can be defined based on histological and molecular criteria. Importantly, certain molecular features of a patient's disease can influence response to current therapies. Animal models that accurately recapitulate the genotypes and phenotypes of each paediatric brain cancer type are rare but essential to evaluate potential new therapies and to introduce novel molecular subgroup-specific treatment protocols to the clinic.

Objective: The XRAD image-guided, small animal radiotherapy (SmART) system combines micro-CT imaging and precision irradiation using millimetre sized X-ray beams. This instrument enables delivery of focal radiotherapy to specific targets, including the cerebral cortex or cerebellum of a mouse. Alternatively, custom collimators can be used to mimic the craniospinal irradiation protocols of the clinic. Our aim was to optimise preclinical radiotherapy protocols in mouse models of medulloblastoma, ependymoma and glioblastoma. More specifically these models represent the molecular subgroups of each brain cancer associated with the poorest prognosis.

Methods: At the onset of tumour-related symptoms, radiotherapy was administered to mice with orthotopically implanted brain tumours in 2Gy fractions to mimic daily clinical dosages. Focal targeting was achieved using a rotating 5mm diameter x-ray beam. In order to minimise irradiation of surrounding normal tissues, various arcs of either 60°, 70° or 80° were evaluated. Whole brain irradiation was performed using a static 1cm diameter beam. Tumour tissue was harvested at 0.5, 2, 6 or 24 hours post-treatment, and immunohistochemical markers of DNA damage and apoptosis were used to evaluate therapeutic efficacy.

Results: A transgenic model of SHH-subtype medulloblastoma (*NeuroD2::SmoA1*) demonstrated exquisite sensitivity to irradiation with significantly increased DNA damage and apoptosis two hours post-treatment ($p>0.0001$). Moreover, reduced tumour size was evident by MRI after five days of treatment (2Gy/day, 10Gy total dose). Immunohistochemical data from orthotopic patient-derived xenograft (PDX) models of Group 3 medulloblastomas also indicated sensitivity to radiation, albeit at a reduced extent relative to the SHH model. Ependymoma and glioblastoma xenografts were minimally impacted by radiation therapy as exhibited by reductions in tumour cell proliferation, but no significant apoptosis. These data serve as baseline information for future experimental protocols that will evaluate combinatorial regimens of focal radiation, conventional chemotherapy, and novel anti-cancer agents.

Conclusion: Transgenic and PDX mouse models are invaluable tools to evaluate new treatment protocols ahead of clinical trials. The XRAD SmART is a unique tool that more accurately recapitulates clinical treatments in a preclinical setting. These experiments will enable rigorous evaluation and selection of viable agents to consider for future clinical evaluation.

Establishment of orthotopic lung and brain tumors for image-guided radiation therapy with photons and protons

R. Bütof (1,2,3)

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Introduction: New irradiation techniques, e.g. proton therapy, have shown promising clinical results. Therefore preclinical experiments investigating their radiobiological characteristics are of high interest. Regrettably, only a minority of promising preclinical results translate into successful clinical trials due to discrepancies of growth patterns and microenvironmental parameters between subcutaneous xenografts and human tumors. Therefore, orthotopic models are assumed to better mirror the clinical situation.

Materials & Methods: For establishment of orthotopic tumor models in nude mice we used various luciferase-expressing human lung carcinoma cells and glioblastoma cells. Different transplantation techniques were tested: injection of small tumor pieces (< 1 mm) of subcutaneous source tumors, percutaneous injection of cell suspensions in matrigel and stereotactically-guided injection of spheroids. Tumor growth was imaged using MRI in the orthotopic brain models, cone beam CT; which is integrated in our Small-Animal Image-Guided Radiotherapy platform (SAIGRT); for orthotopic lung tumors, and optical bioimaging using XTREMEII at different time points after transplantation.

Furthermore, an experimental bedding unit setup that allows for multi-modal, cross-platform imaging was developed.

In addition, a proton-based radiography method for in-line treatment planning and positioning verification of small animals at an experimental proton beam line was implemented. Via dual-energy proton radiography of the same object an enhancement of the signal contribution from either the scattering or energy deposition in tissue of protons has been achieved. Moreover, the acquisition of the respective background images allows for a complete separation of both signal contributions in the following image analysis.

Results: Tumor histology was analyzed via staining with hematoxylin and eosin, and human origin of tumors was verified by a specific anti-human Ki-67 antibody. In both tumor entities we reached take rates between 50 % and 90 % in favor of stereotactic injection methods.

The bedding unit setup meets the demands of various imaging techniques and treatment modalities (photon- and proton irradiation). It consists of a primary bedding unit which is equipped with a breathing mask for inhalation anesthesia, an inlet for warm air and a breathing sensor. The second, peripheral unit comprises a heating module, several sensors and read-out electronics to control and monitor temperature as well as vital signs.

Exploratory experiments with the proton-based radiography method could successfully be applied to reveal internal and external features of phantoms and deceased laboratory mice. The acquired images could also be used for automated registration of proton radiography images to planar X-ray scans of the same object. Dose rates could be reduced to values as low as 50mGy with sufficient image quality.

Conclusion: The established orthotopic tumor models provide the basis to systematically compare radiobiological characteristics between heterotopic and orthotopic counterparts. This is mandatory to further improve the selection of models for preclinical experiments.

The presented bedding unit setup and our proton-based radiography method are considered to be feasible approaches for the precise proton irradiation of subcutaneous and orthotopic tumor models. Further image analysis tools are currently developed to allow for better visualization of the internal structures of the animals.

Inhibition of ataxia telangiectasia related-3 (ATR) improves therapeutic index in preclinical models of non-small cell lung cancer (NSCLC) radiotherapy

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Introduction: Radiotherapy is a major modality in the treatment of localised and inoperable non-small cell lung cancer (NSCLC). Despite significant clinical success, there is an urgent need for novel treatment options to improve therapeutic index, particularly as hypofractionated treatment regimens become increasingly established. Inhibition of Ataxia telangeactasia related 3 kinase (ATR) is emerging as a promising target for combination therapy. In this study, we tested the hypothesis that inhibition of ATR could improve therapeutic index by radiosensitizing NSCLC tumour models without impacting both early (pneumonitis) and late (fibrosis) toxicities in normal mouse lung tissue.

Materials & Methods: AZD6738 was evaluated as a monotherapy and in combination with radiation in vitro and in vivo using NSCLC and bronchial epithelial cell models. Cell line derived xenograft studies were performed using AZD6738 delivered once per day by oral gavage and irradiated with single and hypofractionated radiotherapy. Late toxicities were investigated in fibrosis prone C57BL/6 targeting the upper region of the right lung under CBCT image guidance using the small animal radiotherapy research platform (SARRP). Pulmonary fibrosis was evaluated by longitudinal CBCT analysis over 26 weeks and by collagen staining using Masons trichrome.

Results: AZD6738 specifically inhibits ATR kinase and enhanced radiobiological response in NSCLC models but not in human bronchial epithelial cells (HBECs) in vitro. Significant tumour growth delay was observed in cell line derived xenografts (CDXs) of H460 and A549 cells ($p < 0.05$). The combination of AZD6738 with radiotherapy showed no significant change in lung tissue density by CBCT ($p > 0.5$) and histological scoring of radiation induced fibrosis compared to radiation only controls ($p<0.5$).

Conclusion: AZD6738 is a specific inhibitor of ATR that shows tumour selectively in enhancing radiobiological response in NSCLC models without augmentation of late pulmonary toxicity and further underpins translation towards clinical evaluation in NSCLC radiotherapy.

Tumor reoxygenation and image-guided SBRT for the treatment of murine colorectal liver metastases

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Introduction: Reactive oxygen species are generated in response to ionizing radiation (IR) and produce amongst others irreversible DNA double-strand breaks. This IR-induced cytotoxic effect is less abundant under hypoxia and thus hypoxic cells are more resistant to IR. Hence, reoxygenation of the hypoxic tumor fraction by a combined treatment modality with a pharmaceutical agent is of high interest to reduce the required dose of IR and thereby to further minimize normal tissue toxicity. Here we investigated the combined treatment modality of the novel anti-hypoxia compound myo-inositol trispyrophosphate (ITPP) in combination with IR for the treatment of murine colorectal liver metastases.

Materials&Methods: ITPP was developed as an effector of hemoglobin lowering the oxygen/hemoglobin affinity thereby resulting in an enhanced release of oxygen e.g. in hypoxic tumors. Murine colorectal cancer cells (MC-38) were injected either subcutaneously or orthotopically in the right lateral liver lobe of female C57BL/6 mice. Mice were treated with a previously identified regimen of ITPP (2x3g/kg, neoadjuvant) alone and in combination with a single dose of IR (12 Gy). Tumor volumes were probed by caliper measurements (subcutaneous tumors) and by serial MRI (orthotopic tumors). Tumor detection and irradiation were performed by contrast-enhanced CT and a small animal radiotherapy platform (X-RAD225Cx), respectively.

Results: Treatment with ITPP alone did not reduce the growth rate of subcutaneous tumors as compared to vehicle treatment. However, ITPP in combination with a single high dose fraction of IR (12 Gy) significantly delayed tumor growth in comparison to irradiation alone. An initial IR-dose escalation study in healthy mice was performed with irradiation alone (and in combination with ITPP) of the right lateral liver lobe. While a single dose of 20 Gy was well tolerated, high toxicity (rapid weight loss) was observed in response to a single high dose of 30 Gy. Preliminary results in this murine colorectal liver metastases model revealed a partial radioprotective effect of ITPP in the normal tissue. Efficacy-oriented results against the orthotopic tumors in response to IR in combination with ITPP will be discussed.

Conclusion: Here we demonstrated that the combined treatment modality of ITPP and IR results in a supra-additive tumor growth delay, which is most probably linked to neoadjuvant tumor reoxygenation. Moreover, we showed the feasibility of single high dose irradiation of selective liver lobes performed on a small animal image-guided radiotherapy platform.

Optimizing Radiotherapy-Immunotherapy combinations in preclinical models

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Intro: Immunotherapies have been shown to achieve systemic remission of tumor lesions in patients with metastatic cancers. However, only a minor fraction of patients seems to respond adequately to these therapies due to the existence of rate-limiting immunosuppressive forces in tumors. A growing pile of evidences is revealing that local radiation therapy (RT), applied to patients in specific forms, can turn tumors into *in situ* vaccines, and by itself may also help to overcome some of the barriers to tumor specific immune rejection. The concerted action of radiotherapy with immunotherapy to treat cancers that are non-responsive to monotherapy constitute a novel conceptual platform, but improvements at the clinical front are still very modest. Knowledge from systematic preclinical studies on optimal timing and delivery of RT is still lacking. A proper understanding of the immunomodulatory effects elicited by different radiotherapy schemes will allow implementation of combinatory treatments with maximal clinical benefit to an extended group of cancer patients.

Goal: The specific aim of our project is to map the *in situ* and systemic immuno-responses elicited by local radiotherapy (RT), applied in different schemes in pre-clinical immune competent mouse models. By these efforts, we want to define optimal RT regimens that may be combined with immune checkpoint inhibitors for best outcomes.

Methods: We will establish orthotopic lung tumors in immune-competent mice. Murine lung tumors will be irradiated with high precision using the preclinical image-guided irradiator from PXi that very soon will be installed at our institution. Optical (bioluminescent) imaging of luciferase-expressing tumors will be utilized for quantitative assessments of tumor burden and tumor treatment responses. Irradiated tumors will be collected at different time points and analyzed by *in situ* immunohistochemistry for immune cell subsets infiltration. In a different set of experiments, we will explore "off-target" responses in distant non-irradiated tumors. Different combinations of local radiation schemes with immune checkpoint blockers will be tested in immuno-competent mice carrying subcutaneously induced bilateral tumors.

Preclinical Assessment of Late Gastrointestinal Toxicity with the Addition of Plerixafor to Radiochemotherapy for Cervical Cancer

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Introduction: Plerixafor is a commercially available inhibitor of CXCL12/CXCR4 signaling. We have demonstrated that Plerixafor improves primary tumor control and reduces lymph node metastases when combined with radiochemotherapy (RTCT) using primary tumor-derived cervical cancer xenografts and clinically–relevant treatment regimens. Gastrointestinal (GI) side effects are frequently dose-limiting in patients with cervical cancer treated with RTCT, and the cause of substantial morbidity. The purpose of this study was to evaluate late toxicity on colon-rectal tissue with the addition of Plerixafor to RT or RTCT prior to initiating phase I/II clinical trials in patients with this disease.

Materials&Methods: Late intestinal toxicity was assessed using a gut fibrosis assay with H&E and Trichrome staining of the excised distal colon and rectum 90 days after treatment. A catheter was inserted into the mouse rectum, guided by CT to localize the isocentre. The colo-rectal junction region of the mouse intestine was treated with a single dose of 20 Gy using a 5mm square diameter field; and 360° ARC beam using the XRAD 225, a small animal irradiator. The radiation was given +/-cisplatin (4mg/kg 2 hours prior to RT) or RTCT was given with Plerixafor (5mg/kg/day for 30 days - 2 days prior to and post RT or RTCT). The Radiation injury Scoring (RIS) system was used to quantify late toxicity effects.

Results: The histological analysis at time of sacrifice showed no obvious damage to nearby organs. In mice euthanized 90 days after radiation only, histologic changes consistent with rectal mucosal injury were seen in field in all of the treated mice. Interestingly, reduced damage (lower radiation injury score) in the deeper tissue layers of the colo-rectal junction were observed in mice with the addition of Plerixafor to RTCT compared to RT or RTCT alone, suggesting a protective effect of Plerixafor. Mechanisms for this effect may relate to reduced inflammation or to modification of trafficking of bone-marrow derived cell populations to the irradiated site. There were no observed changes in blood neutrophil levels post treatment (at 30, 60 and 90 days post treatment). Whole GI tract irradiations of the mouse (50Gy; 2Gy fractions) showed minimal toxicity to the intestine, liver and kidney.

Conclusion: There was no evidence of increased GI toxicity with the addition of Plerixafor to RTCT compared to RTCT alone with the possibility of some radioprotection. This builds on our previous work showing improved primary tumor control and reduced metastases with this combination. While further investigation is needed to better understand the molecular mechanisms underlying Intestinal radio-protection, this study provides important preclinical information about the safety of Plerixafor in combination with RTCT to motivate future phase I/II clinical studies in this disease.

Radiation induced liver disease mouse model; a promising step toward understanding of normal liver tissue toxicity

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Background: Radiation of liver tumors has recently shown promising results in clinics. Nevertheless, it applicability is limited by the life-threatening conditions that can result from normal tissue toxicity. The radiation induce liver disease (RILD) usually develops in few weeks to few months after the irradiation challenge, and is characterized by the development of a sinusoid obstruction syndrome and veno-occlusive disease. Although the risk of RILD can be estimated, the underlying mechanism driving this disease has yet to be uncovered. Here we describe our RILD mouse model and subsequent new finding on RILD development.

Methods: Based on CT images obtained using exitron6000 nanoparticles as liver contrasting agent, we irradiate a 5mm diameter liver target with 50Gy using X-Rad SmART small animal image guided irradiation system. Four collection times were realized at 1 day, 6 day, 6 week and 20week post irradiation (PI). Histological evaluation was done based on formalin fixed, paraffin embedded samples using eosin hematoxylin, Masson's trichrome, Sirius red and Evans blue staining. Transcriptomic analysis was perused using two samples of RNA per liver (from the target area and not irradiated area; paired analysis) extracted and purified using columns methods and sequenced single end 100bp from a cDNA library.

Results: Mice submitted to irradiation protocol did not show histological change at day 1 and day 6 PI. At 6 weeks PI centrizonal sinusoid were obstructed by collagen fiber and an increase number of Evan's blue positive ceroid containing macrophage where observed in the target area. 20 weeks PI the observation where amplified in the target area and spread to more distant area that receive smaller irradiation doses. Transcriptomic analysis revealed very different profile at the different time points. After the initial injury, a large number of repair mechanism are ongoing at day 1 PI. At day 6 PI very few processes where found to be different between high and low irradiated samples. Week 6 and 20 PI showed an increasing number of processes ongoing and among them ECM modulator and immune response processes as expected from the histology. Based on the course of the disease, we hypothesise that the key trigger of RILD is the unresolved injury that we could observe at day 6 PI leading to the late fibrosis.

Conclusions: Further characterization RILD marker should allow to better describe RILD mechanistic and further evaluation of prophylactic and therapeutic interventions.

Murine versus human tissue compositions: implications of using human tissue compositions for dose calculations in mice

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Introduction: Elemental tissue compositions are a crucial factor in Monte Carlo dose calculations, since probabilities of particle interactions are influenced by the atomic number *Z*. For low energy photons, photoelectric absorption is the dominant interaction. The probability of photoelectric absorption depends strongly on *Z* and thus, in the case of tissues, the elemental composition. In the field of small animal radiotherapy, it is particularly important to use correct tissue compositions due to the very low energy of the photon spectrum in the treatment beam. It is common practice to use human tissue compositions for dose calculations in mice, since murine tissue compositions are not available in the literature (with the exception of high *Z* materials). However, murine tissues are known to be different from human tissues. For example, murine bone tissues are much more flexible than human bone tissues. The elemental compositions might also be different between human and murine tissues. In this study, the dosimetric implications of using human tissue compositions for dose calculations in mice are investigated.

Materials & Methods: Dual energy CT (DECT) images of 9 female NMRI-nu mice were used to extract several physical properties for different in vivo tissues. First, the effective atomic number and the relative electron density were extracted for each voxel in the DECT images. In the second part of this study, the elemental compositions of the investigated tissues were extracted and to investigate the influence of the tissue compositions on the absorbed radiation dose, mass energy-absorption coefficients were calculated for each tissue.

Results: The mean values for the extracted physical properties were compared to values that were derived from the human reference data. In addition to the mean values, the spread in these values was also investigated as well as the influence of this spread on the mass energy-absorption coefficients. It was found that the natural spread in tissue compositions can have a substantial influence on the absorbed radiation dose which should not be ignored.

Conclusion: Using murine tissue compositions instead of human tissue compositions will improve the accuracy of dose calculations in mice. The spread within tissues should also be taken into account. DECT is an important first step to investigate this spread and to account for differences in tissue composition between different mice and within organs.

Preclinical Characterisation of Radiation Dose-Volume Effects on Early and Late Lung Toxicity

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Introduction: Radiation induced lung toxicity (RILT) is the most common dose limiting toxicity in patients receiving thoracic irradiation, particularly in lung cancer patients who require a high radiation dose (1)(2). Distinction between the early and late phases of radiation response in the lung is arbitrary and overall, RILT represents a spectrum of biological events evolving from the initial stages of early inflammation, symptomatic radiation pneumonitis (RP) through to late stages of fibrosis (RF). The aim of this study is to define the dose and dose-volume relationship of radiation induced toxicity in the lung using mouse models of early inflammatory and late fibrotic responses quantified both in- and outside of the target volume.

Materials & Methods: C3H and C57BL/6J mice were used to investigate early radiation induced inflammatory response and fibrosis respectively. Animals were irradiated with 20 Gy delivered to the upper region of the right lung as a single fraction or three consecutive fractions using the Small Animal Radiation Research Platform (SARRP, Xstrahl Life Sciences, UK). The fractional volume irradiated was determined by cone beam computed tomography (CBCT) prior to irradiation. Histological sections were examined for neutrophil and macrophage infiltration for early effects and Masson's Trichrome for late toxicity. Results were correlated with the Dose Volume Histogram (DVH) parameters calculated for individual mice and changes in the observed CBCT values.

Results: MLD and V10 were found to show a significant correlation with both early and late responses for single and fractionated exposures. Furthermore, the observed tissue responses showed strong dose dependence at later time points. Whilst radiation toxicity was observed outside the targeted volume, responses showed a poorer correlation with the dosimetric parameters suggesting a level of out-of-field response due to non-targeted effects.

Conclusion: Quantitative assessment of normal tissue response closely correlated early and late lung response with clinical parameters demonstrating this approach as a valuable tool to facilitate clinical translation of preclinical studies. This is further enhanced by the non-invasive imaging of these effects and establishing Cone Beam Computed Tomography (CBCT) as a powerful tool analyzing targeted volume effects in normal lung.

Temporally impaired regenerative capacity and increased inflammation and apoptosis in pelvic neurons post-radiation therapy

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Introduction: Prostatic radiation therapy (RT) is presumed to cause erectile dysfunction (ED) through damage to the vasculature and the nerves supplying the penis. This study examined the impact of in vivo prostatic RT on 1) vascular function in the internal pudendal artery (IPA) and penis, 2) ex vivo survival and growth of major pelvic ganglia (MPG) neurons, and 3) neuronal markers of inflammation, regeneration and injury.

Material & Methods: Adult male Sprague-Dawley rats underwent conformal single fraction 22Gy RT using small animal microirradiator to the prostate or sham treatment. Erectile function was assessed by cavernous nerve stimulated ICP/MAP measurement at 2 or 10 weeks post-RT (n=10/grp). MPG were then excised; neurons were dissociated and cultured (n=4/grp). Axon length and branching, and neuronal nitric oxide synthase (nNOS), tyrosine hydroxylase (TH) and TUNEL assay expression were measured 72h. Gene expression of nNOS, TH, beta-tubulin, Schwann cells (glial fibrillary acidic protein; GFAP), markers of nerve injury (activating transcription factor 3; ATF3), regeneration (growth associated protein 43, GAP43), ninjurin-1 (Ninj1, neuroinflammation marker), and Rac-1 (role in dendritic spine elongation) were also measured in irradiated MPGs (n=6/grp). IPA and penises were mounted in myograph. Concentration response curves to phenylephrine (PE), acetylcholine (ACh), and electrical field stimulated (EFS) contraction and NANC relaxation were assessed.

Results: In dissociated MPG neuronal cultures, there was an early increase in neuron length, while branching and nNOS positive neurons decreased ($p<0.05$). Early radiation caused a 2-fold increase in apoptotic neurons ($p<0.05$). However, the gene expression of neuronal markers beta-tubulin, nNOS, TH and markers of injury and repair (ATF3, GAP43) were all unchanged. There was a marked increase in the gene expression of Schwann cell marker GFAP 2 weeks post-RT ($p<0.001$). At 10 weeks post-RT, there was a 20% decrease in neuron length, decreased neuron branching, and 20-30% less nNOS and TH positive neurons ($p<0.05$). Additionally, there was a 2.5 fold increase in the number of TUNEL positive apoptotic neurons ($p<0.05$). Gene expression of nNOS, TH, GAP43 and ATF3 were all decreased ($p<0.05$) while GFAP remained considerably elevated ($p<0.005$). Rac-1 expression was decreased and Ninj1 expression was increased 10 weeks post-RT ($p<0.05$). Interestingly, there was no change in vascular reactivity in the IPA or penises and erectile function was not impaired at either time point following radiation.

Conclusions: While vascular changes were not evident in these animals, pelvic neurons displayed markedly impaired neuritogenesis, neuron survival and decreased nNOS. Schwann cells and neuroinflammatory protein Ninj1 were elevated post-RT and Rac-1 which plays a role in neuritogenesis and branching was decreased. These are potential novel targets to prevent pelvic nerve damage that contributes to RT-induced ED.

Low doses of ionizing radiation induce angiogenesis: benefits, concerns and challenges to health and science

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Introduction: Radiotherapy is widely used to treat human malignant tumors, characterized by uncontrolled growth and capable of invading into adjacent tissues and metastasize. The radiation treatment is classically delivered by fractionated schemes, consisting of a daily small dose, repeated until a potentially curative tumour specific dose has accumulated. The goal of radiotherapy is to eliminate the primary tumor.

However, the healthy tissues near the tumor target volume are also exposed to daily sub-therapeutic doses of ionizing radiation (IR). We and others demonstrate that sub-therapeutic doses of IR comprise a wide range of doses which induce different biological and health effects. High to moderate sub-therapeutic doses of IR are described as promoting human cardiotoxicity and radiation pneumonitis after breast cancer radiotherapy. Strikingly, we showed that the exposure of healthy tissues to moderate to low sub-therapeutic doses of IR promote angiogenesis and consequently metastases development. These findings were validated in human healthy peritumoral biopsies from patients with rectal cancer since the majority of the patients received neoadjuvant radiotherapy. Interestingly, by using a model of experimentally induced hindlimb ischemia, we showed that low doses of IR induce therapeutic neovascularization in vivo. We currently have an ongoing exploratory clinical trial to determine the clinical and molecular effects in "non-option" CLI patients.

Although our published results are focused on endothelial cells, the effect of sub-therapeutic doses of IR in other cells is still unknown and we are particularly interested in the adipose tissue.

Finally, in a multidisciplinary research project, MEDIRAD (Horizon 2020), we established an animal model in which the whole heart of rats will be daily exposed to different doses of IR, during 23 consecutive days, unrevealing the mechanisms by which cardiotoxicity is promoted after radiotherapy.

Materials&Methods: Different experimental models, including human microvascular endothelial cells, 3T3-L1 pre-adipocytes, zebrafish, mice or rats were used. IR was delivered using a linear accelerator operating at a dose rate of 500MU/min. Capillary and collateral densities were assessed after immunohistochemistry and diaphonization, respectively. Capillaries were microdissected using a Zeiss PALM MicroBeamLaser Microdissection System. Biopsies exposed or not to moderate to low sub-therapeutic doses of IR from patients with rectal cancer that received neoadjuvant radiotherapy were collected. Adipocyte conditioned medium was analysed by ELISA and Zymography and used for in vitro (wound-healing) and in vivo (chick chorioallantoic membrane) angiogenic assays.

Results: Our unpublished results show that low doses of IR significantly activate endothelial cells of peritumoral tissues and increase microvascular density. Moreover, the conditioned medium of adipocytes upon exposure of low doses of IR presents higher levels of angiogenic factors and potentiates an angiogenic response both in vitro and in vivo.

Conclusions: Overall, these results provide new insights into the cellular effects of sub-therapeutic doses of IR and a new rationale basis to improve current radiation oncology protocols. Moreover, our work discloses the possibility of using low doses of IR as an innovative, non-invasive and effective therapeutic tool to stimulate angiogenesis in the setting of peripheral arterial disease, with worldwide impact in the treatment options for these patients.

Mild hyperthermia as a localized radiosensitizer for deep-seated tumors – Investigation in an orthotopic prostate cancer model in mice

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Introduction: Mild localized hyperthermia (41 - 43 °C) combined with radiotherapy (RT) has been shown in several investigations to significantly improve tumor control compared to RT alone. A limitation of current preclinical *in vivo* studies on mild hyperthermia (HT)+RT is that most of these have been demonstrated in subcutaneous small animal models. While subcutaneous models are a good starting point, they lack the translatability of orthotopic models because (i) they do not accurately represent the tumor microenvironment, and (ii) they do not consider the potential risks to nearby organs (e.g., rectum, bladder) when administering HT. In this work, we develop an orthotopic prostate cancer model in nude mice and demonstrate proof-of-concept of radiosensitization through the use of external radiofrequency (RF)-induced HT.

Materials&Methods: Under an IACUC-approved protocol, forty athymic male nude mice were inoculated in the ventral lobes of the prostate with luciferase-transfected human prostate cancer cells (PC3). Innoculation was confirmed with bioluminescence imaging (BLI). To obtain a reliable quantitative measure, growth in tumor volume was tracked periodically with ultrasound. The mice were randomized into four arms (1) control (no intervention), (2) HT alone, (3) RT alone, and (4) HT+RT; and the interventions (2-4) were performed after the tumor volume reached 150-250 mm^3. RF-induced hyperthermia was delivered (~30 minutes per animal) using the Oncotherm LAB EHY-100 device. The RF electrode was placed externally, centered over the prostate, and coupled to the skin via a thin layer of ultrasound gel in order to minimize skin burns. The target temperature was 41° C as measured by a thermal probe placed in the rectum. For the RT and HT+RT arms, each animal was imaged using cone-beam CT on the small animal radiation research platform (SARRP, XSTRAHL). Subsequently, a two co-planar field arc plan was created in the vendor-provided treatment planning system (MuriPlan) in order to deliver 12 Gy (single-fraction) to the tumor target.

Results: The overall inoculation success rate was 89.2%, with the remaining animals developing either intra-bladder foci or multi-focal disease, excluding from further analyses. Mean tumor size at randomization was 189.06±12.14 mm^3. The mean time for all groups between inoculation and randomization/treatment was 16.4 days. Mean tumor doubling times in days were (1) Control = 3.9; (2) HT alone = 4.1; (3) RT alone = 27; (4) RT + HT = 36.8. There was significant difference between the normalized nadir volumes for the RT alone (0.76) and the HT+RT (0.40) groups, ($p=0.036$). These early results indicate that mild HT works as a localized radiosensitizer, enhancing the effect of RT, compared to RT alone.

Conclusion: We successfully developed a technique to externally administer mild hyperthermia to deep-seated tumors. Our early results indicate that mild hyperthermia combined with RT may achieve superior tumor control compared to RT alone, without causing significant toxicity to surrounding organs at risk.

Definitive high-dose hypo-fractionated stereotactic brain-sparing irradiation of stage IV canine nasal tumors: a feasibility study and first clinical experiences.

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Introduction: The prognosis for canine nasal tumours with intracranial extension is poor with an expected survival of 1 month with palliation and 6.7 months with irradiation. However, studies regarding stage IV nasal tumours treated with brain-sparing irradiation techniques are lacking. The aim of this prospective study was to evaluate feasibility and efficacy of definitive intent stereotactic radiotherapy in dogs with nasal tumours with massive intracranial extension.

Materials & Methods: Seven dogs with stage IV nasal tumours were treated with high-dose hypo-fractionated stereotactic radiotherapy with VMAT technique. Dose prescriptions were 36 Gy in six fractions to the gross tumour and 30 Gy to lymphatics, on alternate day's basis. Adjuvant treatment included carboplatin. Serial clinical and CT/MRI examination were performed. Disease control and toxicity effects were evaluated according to RECIST and VRTOG criteria. Median survival time (MST) was evaluated using Kaplan-Meier curves.

Results: Six carcinoma and 1 sarcoma were treated. Prescription goals were obtained in four cases with V95%>95% and V107%>2% whereas in 3 dogs V95%=86-90% was accepted to limit maximum brain punctual dose <27 Gy. Two partial response and 5 complete responses were obtained. MST was 9 months. One grade II late brain radiotoxicity and two brain ascending infections were observed. Relapse pathways involves diffuse meningeal and sphenoid invasion.

Conclusion: The initial experiences with the RT regimen adopted indicate a feasibility and effectiveness in modified stage IV nasal tumours. The relapse pathways observed suggest to evaluate alternative adjuvant treatment in dogs treated with stereotactic radiotherapy.

What safety margin for PTV creation should be used in preclinical radiotherapy of mice? A first approximation with a well-known margin recipe.

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Introduction: Today, radiotherapy systems for preclinical research with its on board imaging such as Cone Beam CT (CBCT) enable to apply realistic (similar to human) and precise radiotherapy concepts on small animals. Because of the better localization during the treatment procedure it is possible to apply fractionated schemes and to reduce the irradiated volume to better spare normal tissue. On the other hand it has to be assured that the entire target volume (i.e. the tumor and appropriate margins) receives enough dose, despite possible errors and uncertainties. In human radiotherapy the concept of gross tumor volume (GTV), clinical target volume (CTV) and planning target volume (PTV) is well known. In this work we present how the margin recipe from Marcel van Herk (The probability of correct target dosage: dose-population histograms for deriving treatment margins in radiotherapy. Van Herk M, et al. Int J Radiat Oncol Biol Phys. 2000;47:1121-35) could be applied in this context to derive margins from CTV to PTV for small animals.

Materials&Methods: The formula $m_{PTV} = \alpha^*\sum + \beta^*\sigma - \beta^*\sigma_p$ for fractionated treatments from van Herk's work accounts for the systematic error $\sum$ arisen during treatment planning, the random error σ arisen during treatment execution, and the penumbra σ_p of the treatment beam. In detail, there are terms involved for organ motion, setup-/ registration-error, delineation error and fractionation. The used formula evolved from a dose-population based method to ensure a certain delivered dose (to the CTV) to a certain fraction of the irradiated population. In our example we aim to bring at least 95% of the prescribed dose to 90% of the specimen. As the single variables are dependent on different settings and preconditions like treated area (i.e. for organ movement) and fixation of the specimen (for setup uncertainties) we first discuss how the formula can be used for small animals in general. We will also calculate the margin from CTV to PTV for a specific area of application: for our SARRP device with its on board CBCT, a fractionated (10 fractions) arc treatment (360°, 10x10mm^2 field) of anesthetized immunodeficient nude mice (crl:CD1- Foxn1nu, Charles River Laboratories, Sulzfeld, Germany) with orthotopic pancreatic tumors.

Results: We assumed the following values for calculating the margin in our case (with origin or motivation in brackets): α=2.5 (van Herk et al.), β=1.64 (van Herk et al.), organ motion 0.3 mm (Maleke et al.), setup-/ fusion-error 0.5 mm (own estimation) and the precision of alignment 0.2 mm, delineation error 0.3 mm (own measurement), fractionation 0.46 mm (van Herk et al.), penumbra of the high dose region 1.3 mm (own measurement). With these values we conclude that the needed margin for a PTV (to add to a CTV) is 1.8 mm in our example of a mouse with an orthotopic pancreatic tumor.

Conclusion: A well-known margin recipe from human radiotherapy is transferred to our preclinical setting with mice treated on a SARRP. In the shown example we calculated the margin from CTV to PTV for an arc treatment of mice with an orthotopic pancreatic tumor. We found the margin to be 1.8 mm in this case to ensure an appropriate treatment.

On the determination of planning target margins due to motion for mice lung tumours using a 4D MOBY phantom

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Introduction: Recent improvements and availability of tumour models and precise radiation technology enabled an environment suitable for advanced pre-clinical translational radiotherapy research. For an accurate translation of pre-clinical results into clinical trials it is important that some characteristics are kept consistent throughout the treatment regimens. There is a lack of consensus in the literature on the establishment of the planning target margins, more specifically, the geometrical concept of planning target volume (PTV) for small animal radiotherapy. Thereby, a quantitative approach is proposed to estimate the target margins for mice lung tumours.

Materials&Methods: A 4D digital mouse whole body phantom (MOBY) was used. Either 3 or 4 mm virtual spherical tumours were placed at a number of positions on the right and left lungs. Two breathing curves were used, for a mouse in regular conditions and under anaesthesia, both in different phases, representing a full breathing cycle. The former breathing curve was MOBY's mathematical model and the latter was determined by fluoroscopic imaging of an anesthetized mouse. Using the dedicated small animal treatment planning system SmART-Plan, two types of plans were elaborated, either using 20 equally collimated beams 18^{o} apart or a full arc. Monte Carlo dose calculations were performed for each time point of a breathing curve, each tumour size, tumour position and a set of circular collimators with diameters ranging from 3 to 7 mm. The dynamic mean and time-dependent doses were calculated for the tumour and organs at risk. Spatial uncertainties were estimated and a percentage margin derived.

Results: To ensure that a minimum dose of 95% was delivered to 90% of the volume on a breathing mouse, a margin beyond the tumour was derived considering the standard deviation of the systematic and execution errors, the penumbra width, the standard deviation of the penumbra width and the dose to the tumour and organs at risk. This margin width does not include rotational errors or shape variations and could be used as a lower limit.

Conclusion: Pre-clinical radiation therapy can be meaningfully translated to clinical trials if the dose distribution in space and time can be accurately correlated with the extent of the malignancy. Therefore, a consistent target margin that includes at least breathing motion should be applied in small animal radiotherapy. Different adaptive radiotherapy techniques could further improve the accuracy of volume margins to account for tumour shrinkage or positioning inconsistency, for example.

Evofosfamide sensitizes esophageal carcinomas to radiation without increasing normal tissue toxicity

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Introduction: Esophageal cancer has an increasing incidence and is rarely curable. When treated with radiotherapy, the anatomical location often results in toxicity to surrounding normal tissues such as mucosal tissue and lungs. Most esophageal cancers have hypoxic areas causing resistance to conventional anti-cancer therapies, making them susceptible for treatment with hypoxia-activated prodrugs (HAP). These agents are reduced in the absence of oxygen and form active cytotoxins that kill hypoxic cells. We investigated in vivo whether the HAP evofosfamide (TH-302) sensitizes esophageal carcinomas to radiotherapy. Additionally, normal tissue toxicity of this treatment combination was assessed in short-term (gut mucosa) and long-term (lung fibrosis) toxicity models.

Materials & Methods: To assess therapeutic efficacy, esophageal squamous cell (OE21) or adeno (OE19) carcinoma cells were injected into the flank of immunocompromised mice and treated upon an average volume of 240 mm^3 with 5 consecutive daily injections (QD5) of TH-302 (50mg/kg) or vehicle and irradiated (sham or single dose 10Gy) at day 5. Tumor volumes were measured until four times the start volume was reached. For the normal mucosal short term tissue toxicity, a gut irradiation model was used, while a lung fibrosis model was applied to assess long term toxicity. Tumor or non-tumor bearing mice were injected with TH-302 (50mg/kg) or vehicle (QD5) and subsequently irradiated using a precision image-guided small animal irradiator (XRAD225Cx, PXI). The abdominal area (sham, single dose 8 or 10 Gy) and the upper part of the right lung (sham, single dose 20 Gy) were irradiated with 40-mm or 5-mm square parallel-opposed fields respectively. Damage was assessed 72 hours later by histology and blood plasma citrullin levels (gut) and after 1 year by histology and non-invasive microCT imaging (lung).

bThe combination treatment of TH-302 with irradiation resulted in a significant tumor growth delay in OE19 ($P=0.02$) and OE21 ($P=0.03$) carcinomas, compared to irradiation only with an enhancement ratio of 1.4 for both models. TH-302 monotherapy induced a slight increase in tumor growth delay in line with the histological evaluation of the hypoxic fraction using pimonidazole hypoxia marker. OE19 tumors appeared to be very radioresistant, which was confirmed by in vitro crystal violet cell viability assays. Irradiation resulted in a dose-dependent decrease of crypt survival ($P<0.001$), mucosal surface area ($P<0.01$) and citrullin levels ($P<0.001$) in both tumor and non-tumor bearing animals. TH-302 did not influence the radiation-induced short term toxicity. Results of the long-term toxicity will be presented during the meeting.

Conclusion: TH-302 in combination with radiotherapy induced significant growth delay without enhanced normal tissue toxicity. This indicates that the combination therapy is a promising approach to improve the therapeutic index for patients suffering from esophageal cancer, resulting in better tumor control and quality of life.

Magnetic resonance based small animal radiotherapy in neuro-oncology

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Compared to computed tomography (CT), magnetic resonance imaging (MRI) provides vastly superior soft-tissue contrast. This makes it much easier to visualize lesion boundaries that will result in a much better delineation of the target volume, helping to better irradiate the lesion and avoid surrounding tissue. Therefore, combining MRI with CT data is increasingly used for radiotherapy planning in the clinic. This combined CT/MRI dataset contains both the information required for targeting and for dose calculations. During this presentation, three studies will be presented where MRI and small animal radiotherapy were combined in the field of neuro-oncology.

In a first study, we used a combined CT/MRI dataset to guide the irradiation of brain tumours in a F98 glioblastoma rat model using a micro-irradiator. Contrast-enhanced MRI images were acquired to follow up tumour growth after orthotopic inoculation, to monitor treatment response, and to delineate the target volume during radiotherapy planning. Using multiple non-coplanar arcs the prescribed dose could be delivered to 90% of the target volume, while minimizing the dose to normal brain tissue.

A challenging aspects of small animal CT imaging relates to the radiation dose received by the animals. This might become a very important issue when the therapeutic dose has to be delivered in multiple fractions spaced over time, where each individual irradiation requires a CT for accurate animal positioning. Therefore, in a second study we investigated the feasibility of a MRI-only based workflow for radiotherapy planning of the rat brain that enables both accurate target delineation and accurate dose calculations using only MRI-based volumes. Multiple MRI sequences were used to generate synthetic CT images that could be used for dose calculations, because only one MRI volume was not sufficient to separate all major tissue types (air, soft tissue, bone) in the rat head. The synthetic CT images were sufficiently similar to the segmented CT images that are routinely used for radiotherapy planning on preclinical radiation research platforms. No significant differences were observed between CT and MRI based dose calculations when more complex beam configurations (multiple beams) were used in the dose plan. However, further research is required in the thoracic or abdominal region of small animals, where more tissue classes will be required to allow for accurate dose calculations compared to the rat head.

Finally, discrimination between (high-grade) brain tumor recurrence and radiation necrosis (RN) remains a diagnostic challenge because both entities have similar imaging characteristics on conventional MRI. Functional imaging techniques, such as dynamic contrast enhanced (DCE) MRI or positron emission tomography (PET), could overcome this diagnostic dilemma. A third study will be presented to investigate the potential of DCE-MRI and PET in discriminating high-grade glioma from RN in rats. Induction of RN was achieved by irradiating the right frontal region with 60 Gy using multiple arcs. Results suggested that functional imaging can be used to discriminate glioblastoma (recurrence) from RN.

Combining intravital multiphoton microscopy and image guided radiotherapy to determine the response of tumour vasculature to irradiation

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Background: Radiation therapy affects also the tumour microenvironment; however, its response, including the tumour vasculature is poorly understood. Tumour vasculature itself is generally not reported to be functionally damaged by doses of radiation below ~15Gy, but the status of surviving endothelial cells is still unknown. Efficacy of radiation therapy can be modulated by vascular targeted antibodies, such as antibodies targeting Delta-like ligand 4 (DLL4). Our aim was to delineate the response of tumour blood vessels to radiation therapy and how anti-DLL4 antibodies change vessel structure and function.

Materials&Methods: We used a transgenic mouse model (VE-cadherin-CreERT2-flox-STOP-flox-tdTomato) in which only endothelial cells express tdTomato after treatment with Tamoxifen. An abdominal window chamber was surgically implanted in mice and murine colon carcinoma MC38 tumours induced. The developing tumour vasculature on a single endothelial cell level and perfusion was then imaged for up to 15 consecutive days with multiphoton intravital microscopy. We followed the response of tumour vasculature to single dose (15 Gy) or fractionated (5x3 Gy) radiation delivered by Small Animal Radiation Research Platform (SARRP), and to treatment with anti-DLL4 antibodies.

Results: Intravital multiphoton microscopy revealed distinct patterns of tumour vasculature development for each of the studied treatments. We were able to visualize and quantify sprouting angiogenesis in on a level of single sprout for up to 15 days. When tumours were irradiated, the development of tumour vasculature was arrested for up to 10 days after radiation compared to controls, with decrease in number of newly formed sprouts and elongation of the established sprouts. Treatment with anti-DLL4 antibodies dramatically increased the number of newly formed sprouts and reduced perfusion of blood vessels at later stages.

Conclusion: Our transgenic mouse model in combination with longitudinal intravital imaging and in-house image analysis sets the stage for in vivo visual investigation of the development of tumour vasculature and its response to different treatments including radiation therapy.

The potential of photoacoustic imaging as a predictive and monitoring tool in radiotherapy of head and neck tumours

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Introduction: Radiotherapy (RT) is commonly used for the clinical treatment of cancer. It is well established that cells in hypoxic (low oxygen concentration) regions are more radioresistant. Blood oxygen saturation (sO_2) can be measured non-invasively using photoacoustic imaging (PAI). It has been shown in literature that tissue regions with low blood sO_2 tend to be hypoxic. Radiotherapy can also affect the vasculature and thus change blood sO_2, depending on the dose and fractionation regimes. In particular, hypofractionated RT (>10 Gy/fraction) results in vessel collapse. In this study, we have investigated the use of blood sO_2 measured using PAI as a predictive and monitoring factor in head and neck tumours response to radiotherapy.

Materials & Methods: A head and neck tumour model (CAL^R) was grown in immunosuppressed mice (n=43, female, ~25g). When tumours reached a volume of ~200mm^3 (from caliper measurements), animals were imaged using the multiSpectral optical tomography small animal imaging device (MSOT®, iThera, Munich) at 700, 715, 730, 750, 760, 800, 850 and 900nm. Using a linear regression algorithm provided by iThera (ViewMSOT®, iThera, Munich), we estimated the proportion of HbO_2 and Hb in tumoural blood and calculated the average sO_2 value in 3 central tumour slices.

Following imaging, single fraction radiotherapy was delivered, either at 10Gy (n=13), 20Gy (n=10) or 30Gy (n=10), using a computed tomography (CT)-guided small animal radiation research platform (SARRP®, X-Strahl, Camberley), 225kV, dose-rate=2.5Gy.min^{-1}. The remaining 10 animals were used as controls. Animals were re-imaged 96h after-RT. Tumour growth was monitored up to 60 days after-RT using calipers and treatment response was divided into full-response (tumour regression), partial-response (growth inhibition or delay) or no-response (compared to the control group).

Results: Tumours that had a full-response to treatment had statistically significantly higher average blood sO_2 before treatment, 0.79±0.01 and 0.63±0.08 (unitless), for both 10Gy (n=3) and 20Gy (n=7) treatments, respectively, compared to partial- and no-response (<0.55). For 30 Gy, the initial blood sO_2 had little effect on treatment response, as the high dose caused 8/10 tumours to regress.

Ninety-six hours post-RT, a trend of decreasing average blood sO_2 was measured for full-responders of -13±10% and -15±9% for 10 and 20Gy, respectively. This decrease is possibly due to vascular collapse, which can induce secondary cell death, improving treatment outcome. No-responders showed an increase in sO_2>10% for both 10 and 20Gy cohorts. Neovasculature post-RT has been shown as a negative prognostic factor for large RT doses (>10Gy/fraction). The 30Gy cohort showed increased sO_2 in partial-responders and 6/8 full-responders, possibly because cells were drastically affected causing a reduction in the oxygen demand.

Conclusion: RT doses of 20 and 30Gy resulted in a majority of full-response, while with 10Gy only 3 tumours regressed. PAI measurements showed that tumours with low blood sO_2 responded poorly compared to those with higher blood sO_2, demonstrating the potential of PAI as a predictive tool for RT response. We also showed that PAI can be used as a monitoring tool for RT doses>10Gy, with full-responders showing a decrease in sO_2 and no-responders the opposite effect.

Evaluation of tumor perfusion with IVIM analysis after radiotherapy.

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Introduction: Neoadjuvant radiotherapy (NeoRT) improves tumor local control and facilitates tumor resection in many cancers. The timing between the end of the NeoRT and surgery is driven by the occurrence of side effects or the tumor downsizing. Some clinical studies demonstrated that the timing of surgery and the RT schedule influence tumor dissemination and subsequently patient overall survival (*acta oncol 2006*). Previously, we developed a pre-clinical model demonstrating an impact of NeoRT schedule and the timing of surgery on metastatic spreading (*Oncotarget 2015*). Here, we used functional MRI (fMRI) to record tumor microenvironment modifications after NeoRT. We aim to get non-invasive markers to establish the best timing to perform surgery and avoiding tumor spreading.

Materials&Methods: Based on two NeoRT model, MDA-MB 231 and 4T1 cells, implanted in the flank of SCID and BalbC mice, respectively. We locally irradiated (PXI, X-Rad SmART) tumors with 2x5Gy and then surgically removed them at three different time points after NeoRT. fMRI (9,4T Agilent) were acquired before and after RT. We performed Diffusion Weighted (DW) -MRI every 2 days after RT until surgery. For each tumor, we acquired 8 slices of 1 mm thickness and 0.5 mm gap with an "in plane voxel resolution" of 0.5 mm. For DW-MRI, we performed FSEMS (Fast Spin Echo MultiSlice) sequences, with 9 different B-value (from 40 to 1000) and B0, in the 3 main directions. IVIM (IntraVoxel Incoherent Motion) analysis was performed to obtain information on intravascular diffusion, related to perfusion (*F*: perfusion factor) and subsequently tumor vessels perfusion.

Results: In the MDA-MB 231, we observed a significant peak of *F and D** parameters related to perfusion (60% of the basal value (n=6, $p<0,05$)). The other DW-MRI parameters, ADC and D were stable after irradiation. We observed similar results with 4T1 cells, where *F and D** increased at day 3 (55% of the basal value, n=10, $p<0,05$) then returned to initial level. The difference in timing for the peak of *F* (day 6 *vs* day 3) could be related to the difference in tumor growth according to the cell line (four weeks for MDA-MB 231 cells vs one week for 4T1 cells). When surgery is performed on F and D* peak, we observed a decrease of pimonidazole staining related to hypoxia without any vascular architecture modification (CD31 labeling). Moreover, with the MDA-MB 231 cells we observed an increase of lung metastases: 115% compared to a surgery performed before the peak and 187% compared to a surgery performed after the peak.

Conclusion: We demonstrated the feasibility of repetitive fMRI imaging in preclinical models after NeoRT. We observed a significant modification of MRI vascular parameters (D* and F) after neoadjuvant radiotherapy in two different tumor models. We also performed surgery based on this vascular modification and observed a correlation with tumor metastases. Finally, we demonstrated the feasibility of Image Guided Surgery for decreasing tumor metastases after NeoRT.

Accurate and traceable dosimetry for pre-clinical radiobiological studies

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The ultimate goal of radiobiological studies is to establish and quantify the relationship between the magnitude of biological effect and radiation dose delivered. Contrary to clinical settings (where dose uncertainties are below 3%), dose measurements in pre-clinical studies are frequently inadequate, thus undermining the reliability and reproducibility of the findings. Responsible factors are both technical and logistic. There is a lack of suitable dosimetry protocols to address various specific needs of radiation sources used in the preclinical studies resulting in a variety of approaches being adopted. Particularly for small animal studies, complexity, spatial and mechanical constrains prevent from the confident use of standard methods and technologies. Furthermore, detectors and radiation monitoring equipment are not always appropriately calibrated for the specific radiation quality and usage involves the adoption of large correction factors. Finally, routine quality assurance checks and correct implementation of dosimetry procedures require adequately trained personnel which may not always be available to conventional radiation biology laboratories. According to ICRU Report 24, a change of 7-10% in dose to target volume results in significant change in tumour control probability (TCP). An accuracy of ± 5% in the delivery of absorbed dose has, therefore, been agreed for clinical studies and such constraints should also be applied to the pre-clinical investigations. Funded by the UK government, the National Physical Laboratory is developing the necessary support and infrastructure to ensure that facilities for radiation biology and animal irradiations use dosimetry equipment and methodology which are well characterized, documented and traceable to national standards.

Response of alanine dosimeters to low energy X-rays and small fields

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L-α-alanine Electron Paramagnetic Resonance (EPR) dosimetry is a convenient method for quality assurance (QA) measurements in radiotherapy due to its excellent properties (robustness, nearly water equivalent, nondestructive read-out, low signal fading rate). It has been used for more than 20 years as a transfer dosimetry system mostly for high-energy photon beams where the energy response is very well documented. The rapid development of small animal treatment with kilovolt X-ray sources led us to investigate the use of EPR dosimetry in this field.

The aim of this project is to determine the response of the alanine-EPR dosimetry system in low energy X-rays and small fields (down to the mm range). We will use cylindrical pellets with height 2.8 mm and a diameter of 5 mm (Harwell Dosimeters LTD, Oxfordshire, UK). The dose delivered to the alanine in small fields will be determined using Gafchromic® EBT3 films (Covington, Kentucky, USA) with triple channel dosimetry. The irradiations will be performed on a small animal radiation research platform (SARRP), which has an X-ray source with energies varying between 50 kVp and 220 kVp. The alanine detectors will be placed in a small cubic slab phantom (6 x 6 x 10 cm^3) at different depth in combination with EBT3 films. EBT3 films have been shown by several authors to have a very small energy dependence. They will be calibrated against an ionization chamber at different energies to confirm it. The slabs will consist of water equivalent material RW3. To compare the tissue equivalent property of the solid phantom, a custom-made water tank (10 x 10 x 10 cm^3) will be used where EBT3 film can be placed vertically to measure the depth dose curves. This depth dose will be compared to a depth dose curve measured in the solid phantom.

The procedure we plan to use for the measurements combining alanine pellets and films has already been used in fields of 5x5 cm^2 to 1x1 cm^2 in a PMMA slab phantom for MV beams to assess the response of alanine for small fields. During these experiments, we could show that alanine over responds for fields smaller than 2 x 2 cm^2.

Alanine-EMR dosimetry is a very accurate tool for QA for MV beams in fields larger than 2x2cm^2. However to become a possible dosimeter for quality assurance in small animal radiotherapy, the response should be accurately characterized for low energy photons and for much smaller field sizes. As the pellets have a diameter of 5 mm, the effect of inhomogeneous irradiation of the pellet with beams smaller than the physical dimension of the detector will be investigated.

Investigating the use of the portal imager as a quality assurance tool for the small animal radiation research platform (SARRP)

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Introduction: While there has been increasing use for SA-IGRT systems for pre-clinical radiotherapy research, tools for performing routine quality assurance (QA) have not yet been optimized, nor are they readily available. Robust, efficient, and reliable QA tools are needed to ensure the accuracy and reproducibility of SA-IGRT systems. Several investigators have reported custom-made phantoms and protocols for SA-IGRT systems QA. These are typically time- and resource-intensive, and are therefore not well-suited for the pre-clinical radiotherapy environment where there is limited physics support and routine QA is performed by technical staff. We investigate the use of the in-built electronic portal imaging device (EPID) to develop and validate some common QA tests and procedures. In this work, we focus on the SARRP EPID. However, the methodology and the tests developed herein are applicable to any SA-IGRT system that incorporates an EPID.

Materials&Methods: We performed a characterization of the dosimetric properties of an Xstrahl SARRP's integrated EPID at kilovoltage energies. Specifically: detector warm-up time, radiation dose history effect, stability and short- and long-term reproducibility, output factor, and linearity of the EPID response. We developed a test to measure the constancy of beam quality in terms of half-value layer (HVL) and tube potential using the EPID. The SARRP daily output and beam profile constancy were also verified using the imager. We investigated the use of the EPID to monitor beam targeting accuracy at various gantry and couch angles.

Results: The EPID response was stable and reproducible, exhibiting maximum standard deviation less than 0.3 and 1.9% for short- and long-term, respectively. The short-term reproducibility of the EPID is evaluated over 10 images acquired five minutes apart, while the long-term reproducibility was evaluated using images acquired weekly over an eight-month period. The EPID showed no dependence on response at different gantry angles, with a maximum deviation of less than 0.5%. We found close agreement in output factor measurement between the portal imager and reference dosimeters, with a maximum difference of ≤3% for ionization chambers and ≤1.7% for Gafchromic EBT3 film, respectively. EPID profiles showed quite close agreement with film in both in- and cross-planes, with maximum deviations 3.5% only observed in the in-plane profile of larger collimators. The EPID response was linear with exposure settings (mAs) at all energies, and highly sensitive to small changes in dose and dose rate. Notably, there was a close relationship between the detector response vs mA slope, and the accelerating potential, allowing an independent verification of kVp stability based solely on EPID response. The maximum displacement of the central axis of the x-ray beam (due to sag) was 1.13 ± 0.1 mm at gantry 135°/couch 0° and 1.92 ± 0.1 mm at gantry 0°/couch 90°.

Conclusion: We demonstrated that the SARRP EPID is a stable, and convenient tool. We proposed tests to perform routine SA-IGRT QA such as verifying the constancy of beam quality (HVL and kVp), output, profile and target localization accuracy.

Dosimetric consequences of the use of thin filters in modern image-guided small animal irradiators

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Introduction: Recent advances have led to the development and a rapid rise in the utilization of image-guided small-animal irradiators. Two models are commercially available: the SARRP from Xstrahl and the smART from PXi. These irradiators are capable of much more precise delivery of radiation compared to traditional cabinet-style biological irradiators. A traditional cabinet-style irradiator is typically operated at 300-320 kVp, using a thoraeus filter comprising a thick component of Tin, which is high-Z and filters the lower-energy contributions of the beam. In contrast, the SARRP uses 220 kVp/0.15 mm Cu and the smART 225 kVp/0.30 mm Cu. This produces comparatively very lightly filtrated beams whose photon spectrum is much more weighted towards low-energy photons.

In this study, we perform Monte Carlo calculations of absolute dose rate, relative percent depth dose, and radiation biological effectiveness (RBE) of these beams to assess their sensitivity with slight changes in thickness of their filters.

Materials&Methods: We modelled an XStrahl SARRP using a validated Monte Carlo package (Penelope), which was used both for macro and micro-dosimetric simulations. By varying the thickness of the copper filter in the path of the beam from 0.1-0.3 mm Cu, we produced various spectra of varying beam qualities (0.45 mm Cu to 0.90 mm Cu) using SpekCalc. These spectra were used to calculate percent depth dose profiles and absolute dose in water, as well as micro-dosimetry calculations to simulate DNA damage for RBE evaluation. RBE was calculated for 0.15 and 0.30 mm Cu filters, as these correspond to the XStrahl SARRP and the PXi smART, respectively. To compare with a typical biological irradiator, the RBE was also calculated for a PXi X-Rad 320 unit using a 320 kVp beam filtered using 0.75 mm Sn+ 0.25 mm Cu + 1.0 mm Al.

Results: The calculated value for RBE was 2.3 % higher for the SARRP and smART compared to the X-rad 320 cabinet irradiator, and 7. Absolute dose and percent-depth dose at 2 cm depth was found to vary at the rate of -1.8%/0.01 mm Cu and +0.4%/0.01 mm Cu of filter thickness, respectively.

Conclusion: We investigated the influence of thin-filter design on absolute dose, percent-depth dose, and RBE of image-guided small animal irradiators. Compared to the majority of radiation biological studies conducted using heavily filtrated cabinet irradiators, the much thinner filters of image-guided small animal irradiators introduce changes in RBE. Furthermore, the beam quality and absolute dose are highly dependent on precise machining of the filter, where a change in $1/100^{th}$ mm can make a measureable change to the dosimetric properties of the beam. This implies that there may be higher differences in dose and dose rates between different units than in conventional cabinet-style irradiators. These results emphasize the need for careful unit-specific commissioning of image-guided small animal irradiators, particularly when the treatment filter may be damaged or replaced.

New approaches for studying radiobiological effects of kilovoltage X-rays in vivo and in vitro

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Introduction: X-ray microbeam radiation therapy (MRT) as a novel tumor treatment strategy delivers high doses in spatially fractionated X-ray beamlets promising reduced normal tissue toxicity, compared to conventional irradiation, and an improved tumor control. Radiobiological studies of MRT are mainly performed at synchrotron radiation facilities with high costs and space requirements. The Munich Compact Light Source (MuCLS) as a laboratory-sized and cost-effective source based on inverse Compton scattering of infrared laser photons produces low keV X-rays with a short penetration depth. However, the standard method for assessment of treatment efficiencies is not well-suited for short penetrating X-rays since it measures the growth delay of subcutaneous tumors in the hind leg of small animals. Therefore, we successfully developed a setup for a growth delay study in a tumor-bearing mouse ear model to investigate MRT at the MuCLS. In addition, we established a protocol to isolate tumor cells from irradiated tumors to evaluate radiobiological effects on a cellular level.

Materials & Methods: The MuCLS was operated at 25 keV X-ray energy with dose rates of up to 5 Gy/min using an additional collimating polycapillary optic. A Tungsten-Air collimator was inserted to subdivide the homogeneous X-ray beam into 50 µm wide and 350 µm spaced microbeams. We implemented the mouse ear tumor model with a human head and neck cancer cell line, FaDu, suspended in extracellular matrix and subcutaneously injected into the right ear of NMRI (nu/nu) mice. When tumors reached a diameter of about 2 mm they were irradiated with a dose of 3 and 5 Gy. Tumor growth delay was determined with a caliper over a follow-up period of 30 days and compared between MRT, homogeneous and control mice. Animals were sacrificed when tumors reached the 15-fold initial volume. A single tumor cell suspension was prepared from excised tumors to analyze cell survival by colony formation assay and stable chromosomal aberrations by two-color fluorescence in-situ hybridization.

Results: We successfully installed a setup at the MuCLS which allows irradiation of tumors in small animals and implemented a mouse ear tumor model. Homogeneously irradiated tumors exhibited a growth delay of 9.7 days after 5 Gy irradiation compared to control mice. Tumor growth was not delayed after homogeneous irradiation of 3 Gy and MRT of 3 and 5 Gy. Preliminary data show an increased radiosensitivity of tumor cells originating from homogeneously and MRT-irradiated tumors compared to control tumor cells.

Conclusion: This innovative approach allows the irradiation of tumors in a mouse ear model at a compact synchrotron X-ray source, the MuCLS. Homogeneous irradiation induced a tumor growth delay after 5 Gy irradiation, whereas MRT with doses up to 5 Gy did not, which indicates that higher doses are necessary for tumor growth inhibition after MRT. Furthermore, we successfully validated a protocol for tumor cell isolation allowing the analysis of radiation-induced effects.

Small Animal IMRT using Double-Focused Sparse Orthogonal Collimators

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Purpose: To better translate preclinical research to clinical application, small animal radiation needs to closely simulate human radiotherapy which is predominantly intensity modulated radiotherapy (IMRT) conforming dose to the target. Straightforward translation of human IMRT method has been shown impractical for small animals. We design and manufacture a novel dose modulation device and the corresponding planning system to overcome the challenge.

Methods and material: With delineated target and avoidance structures, an optimization problem was formulated including a L2 norm fidelity term and a L1 regularization term. The L2 norm penalizes target dose deviation from intended dose and any dose to the avoidance structures. Beamlets with the resolution of 0.5 mm^2 were calculated for 180 coplanar beams around the animal using convolution/superposition of Monte Carlo calculated kernels. Beam orientation and fluence map optimization was performed using column generation. The resultant fluence map was decomposed to rectangular apertures and the coefficients of the decomposition were penalized by the L1 norm to minimize the number of rectangles. Rectangular aperture optimization (RAO) was used to create IMRT plans with highly concave targets in the mouse brain. The plan quality including target dose homogeneity, dose gradient and dose conformality was compared to that using a hypothetical miniaturized multileaf collimator (MLC). Further RAO plans were created for a brain tumor bearing mouse based on the tumor PET signal intensity.

The optimized plans were deliverable using a double-focused sparse orthogonal collimator (SOC) with two orthogonal sets of leaves, with two double-focused leaves in each bank. An Arduino board will control the stepper motors driving each leaf at over 4 cm/sec with 0.02 mm resolution. The SOC design features an adapter to securely attach to the X-ray tube head of the PXI X-RAD SmART system.

Results: In the first test using RAO, doses conforming to the concave targets in the brain were achieved with rapid fall off outside of the target. Both the dose gradient and conformality were superior to the MLC plans. The target dose homogeneity was also substantially better than MLC plans. In the second tests, simultaneous integrated doses conforming to the PET active volume was created. RAO was able to provide positive or negative boost to the hyper-metabolic volume. In these plans, the mean number of deliverable apertures between all of the plans is 9.7 ± 0.84 segments per beam. The intensity modulation using copper SOC leaves and lower 80 kVp was demonstrated while tungsten leaves for 225kVp are being tested.

Conclusion: With advanced optimization algorithm, complex IMRT plans can be created using simple SOC, which is practical for miniaturization due to its fewer moving components and high dose modulation resolution.

Spatially-modulated proton minibeams on the UW radiation platform for preclinical in vivo research

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Introduction: The University of Washington (UW) has developed the first image-guided experimental small animal platform to investigate radiobiological effects of proton beams. This talk provides an overview of the cyclotron-based facility, with a focus on spatially modulated proton minibeams generated with a multislit collimator. Most experimental results to date with highly modulated beams have been obtained on synchrotron generated X-ray microbeams. An approach is presented that accounts for different biological effects of different beam types/energies as well as different spatial geometries of the beams. The aim is to account for inter-institutional differences in the beams and ultimately to separate known and unknown biological mechanisms of action.

Materials & Methods: The UW 50.5 MeV proton beamline was fully characterized by means of TOPAS Monte Carlo simulations, both dosimetrically and with respect to relative biological effectiveness (RBE). A comparison of the RBE for nominal proton beam energies in the range 30-109 MeV is presented as a basis for inter-institutional comparisons of minibeam experiments. The equivalent uniform dose (EUD) concept, based on the well-established linear (LQ) quadratic model, is introduced as a means to compare spatially-modulated and uniform beams.

Results: A spectral analysis of the UW proton beam line reveals a near monoenergetic 45 MeV beam on exit with a pristine Bragg peak (PB) at 17mm depth and a RBE for DNA double strand break (DSB) induction ranging from 1.04 on entrance, 1.4 at the tip of the BP and 1.8 a few mm distal to the BP. While the RBE on entrance is comparable with proton beams reflecting other institutions in the range between 30-109 MeV (1.0-1.1), at the BP it varies by ~30% (1.2-1.6). The UW multislit collimator had minimal impact on the RBE at the BP but increased the RBE in the valleys by ~5%. The EUD calculations for different geometries reveal a strong dependency on the cell sensitivity and total dose. For moderate modulation and standard fraction doses, the EUD is comparable to the mean dose, regardless of cell sensitivity. For highly modulated beams and large dose per fraction, the EUD is much lower and tends towards the valley dose, especially for radiosensitive cells.

Conclusion: The UW small animal proton beam platform is a unique tool for proton beam radiobiology research. For spatially modulated proton beams, large differences between the physical mean dose, RBE-weighted dose and EUD are revealed. While the presented approach has known limitations, it provides a solid framework based on established metrics to account from well-established biological mechanisms among beams and target scanning patterns. Both RBE and EUD corrections are strongly recommended when designing and reporting the results of comparative studies of uniform and spatially fractionated dose distributions. This will facilitate fair comparisons between different type beams and interinstitutional experimental setups as well as better quantification of the effects that may not be adequately described by DSB induction and the LQ cell survival model. Experimental verification of the theoretical concepts are needed.

Establishment of small animal irradiation at University Proton Therapy Dresden

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Introduction: It is a common practice to use a fixed relative biological effectiveness (RBE) of 1.1 when planning treatments and analyzing outcomes for proton therapy. In contrast, a multitude of *in vitro* experiments demonstrate variable RBE values. Also, some clinical evidence of RBE variability is emerging, especially at the distal edge of proton treatment fields, showing increased risk of normal tissue complications. However, only a limited number of *in vivo* trials have been performed to confirm such results. This contribution presents an irradiation setup to study adverse effects in mouse brains induced at proton field edges.

Methods: The mouse is fixated (teeth, ears) in a closed sterile 3D printed holder specifically designed for CT and MR imaging as well as for irradiation with X-rays and protons. Target delineation based on CT and MR imaging can be performed before irradiation. Image-guided positioning of the target volume is achieved by proton radiography with the mouse in treatment position.

Results: In a first brain toxicity study, the distal edge of a laterally collimated clinical proton field (150 MeV) will be positioned in the proximal hemisphere of the mouse brain by inserting polycarbonate plates in front of the mouse holder. For different beam settings, dose distributions in treatment position were obtained with radiochromic EBT3 films placed in plastic phantoms within the mouse holder. Variation of the proton beam range and lateral shape with the amount of decelerating material and collimator size, respectively, were analyzed and used to build a proton beam model. The beam intensity, measured with an ionization chamber, was correlated with the EBT3 film dose measurements at treatment position. This allows for a controlled irradiation of the brain volume with predefined and absolute dose values.

Results and Conclusion: All requirements for systematic proton irradiation experiments *in vivo* are established at the University Proton Therapy Dresden including target volume delineation, mouse positioning, and dosimetry. First experiments comparing brain toxicity after proton irradiation of one hemisphere relative to photon treatment are in progress.

Exploring the capabilities of a clinical proton beam with an adaptive aperture for pre-clinical research

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Introduction: To explore the different capabilities of a clinical proton beam for irradiation of small animals using its unique commercially available adaptive aperture and energy selection system.

Materials&Methods: The proton beam nozzle was modelled using TOPAS, a MC particle simulator. The nozzle includes the Adaptive Aperture (AA), consisting of moving leaves which travel from spot to spot, to shape the beam downstream of the nozzle's exit window. Its energy selection system, slabs of polycarbonate interposed in the beam following a given pattern, was also modelled. The MC model was validated with experimental data. The possible energies of said system range from 0 to 230 MeV, allowing the use of low energies (e.g. < 60 MeV) to irradiate small volumes, such as mice. An extra solid collimator made of brass was modelled and placed downstream of the nozzle's exit window to attenuate the protons passing outside of the AA module. Simulations for a 51.82 MeV beam were performed in a water volume, placed downstream to the external collimator, to evaluate the Bragg Peak position, peak width, spot size, fluence and scatter. Simulations were performed for different AA openings, in which the leaves can completely close (in the horizontal direction), but have a minimum opening width of approximately 5 mm in the vertical direction. AA openings of 5, 10, 15 and 20 mm^2 were used to investigate the optimal opening size and the possibility to give a homogeneous dose to volumes.

Results: The non-collimated 51.82 MeV beam presents a spot size of 1.902 cm, showing the impossibility of irradiation small volumes without collimation for this clinical system. Also, due to the small dimensions of the AA module (4.5 cm x 6.5 cm when completely closed), an external collimator is required to avoid dose leakage outside the AA. The use of the AA aperture of 20 mm^2 reduced the dose to the water volume to 0.5% with respect to the dose without collimation. For the same AA opening, an area of 12.5 mm^2 receives a homogeneous dose.

Conclusion: The use of a clinical proton accelerator, which allows the production of low energy proton beams, has the potential of irradiating small animals with a single beam spot, by means of using the adaptive aperture and an external collimator. Fundamental limitations to the proton fluence distribution of this configuration cannot be overcome, due to the scatter caused by the plates of the energy selection system. Further research is currently being done to find the optimal AA opening and external collimator that results in a homogeneous dose to a given volume.

Design and commissioning of an image-guided small animal radiation platform for proton radiobiology research

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Introduction: There are many challenges with proton radiobiology research in small animal models. We describe our experiences in the development, testing, and commissioning of an image-guided proton system for in vivo, pre-clinical radiobiological research applications with small animals using a commercially available small animal research platform.

Materials & Methods: A 230 MeV proton beam from the clinical cyclotron at the Roberts Proton Therapy Center at the University of Pennsylvania was modified to produce collimated beams down to a 5mm x 5mm square field. The beam is modulated to ranges useful in small animal applications and coupled with a commercial Small Animal Radiation Research Platform (SARRP, Xstrahl Inc). PDD curves and lateral beam profiles were measured for a range of available energies. Ionization chamber and GafChromic™ film measurements were performed and benchmarked for dose output and alignment verification. A comprehensive quality assurance procedure was developed and implemented to ensure consistent and accurate radiation dose delivery using film and semi-automatic image analysis using MATLAB. Preliminary mouse models were irradiated using the image-guided proton radiation system.

Results: The integrated platform has been commissioned to deliver beams with a Bragg peak with a minimum range of 4mm and a maximum range of 30mm in water with a dose rate of 2 Gy/min. The system is capable of delivering a unique SOBP for each experiment by layering pristine Bragg peaks, with a current maximum range of 30mm and maximum width of 25mm. The alignment of the proton beam with the SARRP has been found to have agreement within 2 mm, and the CBCT and robotic animal stage capabilities of the SARRP ensure accurate proton delivery within 1mm. A custom phantom was created for the quality assurance protocol to ensure reproducible alignment of the platform as well as verify daily output. Preliminary mouse models have shown the system is capable delivering a SOBP with accuracy.

Conclusion: We show the feasibility of a proton beam generated by a clinical system integrated with a commercially available small animal x-ray image-guidance system. This will enable in vivo investigations of radiobiological effects in proton beams, in vivo comparison studies between photon and proton beams, and investigation into novel proton treatment methods.

Multifunctional agent for breast cancer radiotheranostics

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Introduction: Breast cancer (BC) is the most frequently diagnosed cancer in women worldwide. The estrogen receptor (ER) a ligand-inducible transcription factor that belongs to the nuclear receptor family, is expressed in approximately 75% of BC cases. Therefore, ER is an important biomarker for prognosis and guiding treatment of BC and is a target for molecular imaging and radionuclide therapy. Regardless of the enhancement in the survival rate of these patients due to new clinical strategies, there is still a need for more effective and personalized treatments to improve BC management. Theranostics is particularly suitable for that purpose as the ER status profiling acquired by imaging can be used to targeted treatment. Thus, one approach that combines into a single ^{111}In-hybrid compound, a chelator simultaneously conjugated to an ER ligand and an antitumor agent for dual targeting of BC cells might enhance therapeutic efficacy. To achieve that goal we have prepared a hybrid compound containing two different biological targeting moieties, an ER ligand (LXXLL based peptide) conjugated to a DOTA derivative functionalized with an antitumoural agent (the DNA intercalating agent, acridine orange, AO) aiming the selective delivery of AO into ER+ breast cancer cells. The bifunctional probe (ER3AO) was radiolabelled with the Auger electron emitter Indium-111 that has simultaneous emission of gamma radiation.

Materials&Methods: Radiolabelling of ER3AO with indium-111 was performed at 95ºC, in pH=5 acetate buffer. Radiochemical purity and in vitro stability of the radiolabelled compound was evaluated by HPLC. Cellular uptake of ^{111}In-ER3AO was assessed in MCF-7 (ER+) human breast cancer cells. The subcellular localization, in particular the internalization of the radiolabelled compound into the cell nucleus was also evaluated. The ability of ^{111}In-ER3AO to induce DNA damage in vitro was tested by incubation with double-stranded plasmid DNA for 140 hours. Biodistribution was assessed in female mice with MCF-7 xenografts and microSPECT imaging studies are underway.

Results: The double vectorised chelator ER3AO was successfully radiolabelled with ^{111}In with high radiochemical yield and purity and its chemical identity was ascertained by comparing its HPLC profile with that of the inactive Indium complex. The ^{111}In-ER3AO demonstrated a fast and high cellular uptake reaching a maximum value of almost 21% after 1 h of incubation. The labelled compound revealed a high rate of internalization in the nucleus that increases over time. Additionally, ^{111}In-ER3AO causes DNA damage by a combination of direct and indirect mechanisms. Biodistribution studies in tumour-bearing animal model revealed fast blood clearance and uptake in ER rich organs and tumours.

Conclusion: The ^{111}In-ER3AO is able to localize in the nucleus of BC cells and of inducing DNA damage in vitro. These findings along with the favourable biodistribution profile in tumour animal model with fast clearance from main organs and accumulation in target tissues suggests that this ^{111}In-hybrid compound could have potential as theranostic agent.

A novel preclinical data management platform to improve translational research

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Introduction: Preclinical biological research is mandatory for developing drugs to investigate the toxicity and effect of the potential drug and develop and characterize animal models. In radiobiological research, the recent technological advances have produced equipment which can precisely irradiate targets ìn animals and use advanced onboard image-guidance, mimicking a clinical environment. This fact is shifting the opinion in the field towards an approach that new treatments also should be tested on toxicity and effect prior to clinical introduction as well. The goal of preclinical studies in general is to translate the result to clinical applications/trials. However, one of the main impediments is a proper data management platform for preclinical studies. Such a platform should be capable of: 1) handling large volumes of data from many different sources; 2) integrating with preclinical systems (e.g. treatment planning systems); 3) supporting plug-ins for outcome prediction models; 4) managing the workflow of animal experiments, 5) reporting of the study results 6) track the animals during preparation and the entire project. The work presented here describes the development of a data management platform for pre-clinical research.

Materials & Methods: A preclinical research platform is developed implementing an adapted version of the clinical DICOM RT 2nd gen. course model. Furthermore the newly defined DICOM suppl. 187 for storing small animal acquisition context is supported is supported in the platform. A modular platform was developed entailing: 1) a study workflow manager, researchers should be able to create a custom study time-line to plan and prepare their experiment; 2) a data manager keeping track of all the data produced throughout the study; 3) a storage manager, keeping track of the physical location of the data produced. For each study, a simple workflow consisting of the following steps: 1) CT image acquisition; 2) treatment planning; 3) treatment delivery was created. The image acquisition and treatment delivery was executed on an image-guided precision irradiation platform (X-Rad 225Cx; PXi, CT, USA), while the treatments plans were produced by SmART-ATP (PXi & SmART Scientific Solutions, Maastricht, NL).

Results: For a sample workflow, the planning CT and bioluminescent images and the treatment planning data (dose and structures) of SmART-ATP are stored in the systems data warehouse. Furthermore, from the X-RAD 225Cx the delivery log files are stored in the systems data warehouse as well.

Conclusion: The developed preclinical data management platform supplies an infrastructure for preclinical research enabling data warehouse functionality capable of storing data from various sources. Furthermore, the architecture will support the analysis of large data-sets stored in the platform using image processing plug-ins of custom models with the goal to create a foundation to translate preclinical results into clinical trials and make them available globally. The platform will facilitate data exchange between researchers.

Use of the liquid fiducial marker BioXmark for high-precision radiotherapy of an orthotopic pancreatic tumor mouse model

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Introduction: High-precision radiotherapy (RT) especially with high single doses requires precise positioning. Fiducial markers in combination with onboard imaging are excellent tools to support this.
The purpose of the study is to establish a mouse model for image-guided radiotherapy (IGRT) in pancreatic cancer using the liquid fiducial marker BioXmark (Nanovi, Denmark) in combination with high-precision small animal RT.
Materials&Methods: In an animal based cancer model different volumes of BioXmark (10-50 µl), application forms, imaging modalities (cone beam computer tomography (CBCT) incorporated in the SARRP (Small Animal Radiation Research Platform) and small-animal micro-CT Scanner (SkyScan)) and subsequent irradiation with SARRP System were analyzed to derive recommendations for the use of BioXmark.
Results: The use of even small volumes (10 µl) of BioXmark was sufficient to be detected by CBCT (SARRP and Skyscan). However, larger volumes (50 µl) led to hardening artefacts. The position of BioXmark was monitored at least weekly by CBCT and was stable over 4 months. BioXmark was shown to be well tolerated, no changes of the physical condition or toxic side effects were observed in comparison to untreated control mice. BioXmark enabled an exact, unique reproducible fusion with the original treatment plan with less hardening artefacts and minimized the application of contrast agent for the fractionated RT setting.
Conclusion: For the first time an orthotopic pancreatic tumor mouse model was established for an image guided high-precision RT with the use of a fiducial marker. BioXmark was successfully tested in a preclinical tumor mouse model and provides a perfect basis for an improved imaging in high-precision RT. BioXmark performance enables a unique application and optimal targeted precision to the gross tumor volume in fractionated RT treatment options. Therefore, preclinical trials evaluating novel fractionation regimens and/or combination treatment with high-end RT can be performed

Optimization of X-Ray Dark-field microCT for Murine Imaging Studies of Radiation-Induced Lung Fibrosis

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Introduction: The first in-vivo dark-field microCT setup is now being used several years in the investigation of lung disorders in murine models. It has been shown that dark-field radiography and tomography can provide local information on developing lung diseases at early stages. In our imaging study we focus on radiation-induced lung fibrosis as it is a severe side-effect after radiotherapy of thoracic tumors. Yet, the performance of this prototype and its efficiency with respect to acquisition time require further improvement. Our goal is to balance out the amount of projections, the amount of phase steps and the exposure time while also minimizing the total time needed for one tomography.

Materials & Methods: The SkyScan 1190 microCT (Bruker, Belgium) is a preclinical imaging device that allows for x-ray dark-field imaging. It is a grating interferometer that employs a phase-stepping procedure to provide conventional absorption-based and dark-field contrast simultaneously. State-of-the-art gratings can be produced on a thinner substrate allowing for a higher X-ray flux. A new set of gratings increased the X-ray flux by 30% and from that emerged new tomography protocols that allow for a reduction of the total acquisition time. The imaging study contained twelve C57BL/6 mice in two groups. One was a control group and the other one contained six mice that received 20 Gy of irradiation on the whole right lung. Local irradiation was performed with 220 kV X-rays using the Small Animal Radiation Research Platform (SARRP, Xstrahl Ltd, UK). Two opposing anterior and posterior oblique field were used to avoid the heart. Dark-field and absorption-based radiographies and tomographies were acquired before irradiation and then again after 24 weeks using the SkyScan.

Results: The results show that the time necessary for dark-field tomography can be reduced below 30 minutes while still maintaining a decent quality in both contrasts. An increased X-ray flux and a suitable detector could even reduce the necessary time further thus providing a fast method to assess lung injuries at an early stage. Our imaging study showed that 24 weeks after irradiation lung damages are clearly visible in dark-field radiography and that the ratio (mean value of the right lung)/(mean value of left lung) deviates about 20% from that of a healthy lung. In absorption-based radiography this deviation is only about 3% at the same point in time. As tomography is expected to more sensitive than radiography the detection might also be possible before the 24th week. Finally, the imaging results were verified using histopathology of lung tissue.

Conclusion: Dark-field imaging shows structural changes of the lung earlier than absorption-based imaging. It is not only quantitatively more sensitive but also shows local changes of the lung structure more distinct and thus provides valuable information on the state and location of the injury. With the improved setup small-animal dark-field tomography can be performed in less than 30 minutes in the future.

Multifunctional Bioconjugated Gold Nanoparticles for Cancer Theranostics

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Introduction: The rapid advance of nanotechnology can play a pivotal role in the design of new pharmaceuticals for cancer treatment. In this field, gold nanoparticles (AuNPs) have emerged as attractive tools due to their appealing physico-chemical properties. In particular, their radiosensitization capabilities make them very suitable as radiosensitizers to improve radiotherapy outcomes. Additionally, AuNPs can also be explored as multifunctional platforms for targeted-delivery of radionuclides and chemotherapeutic drugs for theranostic applications. Herein we report on the synthesis, characterization and biological evaluation of AuNPs decorated with a chelator for coordination of medically relevant trivalent metals (^{67}Ga, Gd) and a bioactive peptide (Bombesin, BBN) for targeted delivery to the gastrin releasing peptide receptor (GRPr) overexpressed in certain cancer cells (e.g. prostate). The biological studies included the assessment of their cellular uptake and radiosensitizing properties using GRPr-positive human tumor cells, as well as biodistribution studies in mouse xenografts.

Materials&Methods: AuNPs were initially synthesized based on the method described by Brust *et al.* by reduction of $HAuCl_4$ with $NaBH_4$ in the presence of a thiolated DOTA derivative (TDOTA) as a stabilizer molecule and chelator for trivalent metals. Loading of the bioactive peptide into the AuNPs was done afterwards by reaction with a thiolated BBN derivative. Gd coordination was done by reaction of the AuNPs with $GdCl_3$; their radiolabelling was done by reaction with $^{67}GaCl_3$. Relaxivity studies were performed using the Gd-containing AuNPs. The affinity of the AuNPs towards the GRPr was done by competitive binding assays using the human prostate cancer PC3 cell line. Cellular internalization studies were done in the same cell line using the ^{67}Ga-labelled AuNPs. The biodistribution of ^{67}Ga-AuNP was assessed in PC3 xenograft balb/c mice after intravenous administration. Finally, their radiosensitization properties were evaluated by studying the clonogenic survival of PC3 cells incubated with the AuNPs and treated with □□and X-ray radiation.

Results: Small core (4-5 nm) AuNPs stabilized with TDOTA and decorated with a BBN analog were successfully synthesized and demonstrate a significantly higher affinity towards the GRPr compared with the ones without peptide. Some of these AuNPs were coordinated to Gd and relaxivity studies of the resulting nanoconstructs displayed T2 contrast imaging capability. The AuNPs were radiolabeled with ^{67}Ga and cellular uptake studies performed showed high internalization for the BBN-containing nanoparticles, and their biodistribution indicated some significant tumor uptake. Radiosensitization studies showed a higher cell death for cells incubated with the AuNPs.

Conclusion: The resulting AuNPs described in this work demonstrated a good affinity towards the GRPr and their biodistribution profile indicated that these nanoparticles can accumulate in tumors following intravenous administration. Relaxivity studies of the Gd-coordinated AuNPs showed that these nanoparticles display suitable T2 relaxivity properties. Finally, it was verified that the AuNPs were capable of enhancing cell death when incubated in irradiated cells. These promising results demonstrate that these multifunctional AuNPs have potential as multimodal agents for image-guided radiotherapy of cancer.

μ-RayStation 5: Expanding functionality of a clinical treatment planning system towards application for image-guided small animal radiotherapy

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Introduction: Modern preclinical radiotherapy devices have been adapted to small animal and target size constraints: beam size, targeting accuracy and image resolution were scaled-down; and beam energy was reduced from MV to kV. They allow mimicking of patient workflow for clinical image-guided radiotherapy (IGRT). A team at Oncoray Dresden has developed a house-made Small Animal Image-Guided Radiation Therapy (SAIGRT) system and a team at Nantes has acquired the XRAD225Cx from PXi Inc. Both systems consist of a stationary unit incorporating a 3D computerised animal stage positioner and a 360° rotating arm holding a 225 kV X-ray tube, a collimation system for beam shaping and a flat-panel detector for X-ray imaging. However, only simplified treatment planning has been applied so far, in lack of a full 3D treatment planning system (TPS).

Materials & Methods: *RayStation 5* is an advanced, clinical TPS by RaySearch Laboratories, which works across different external beam radiotherapy devices (e.g. linear accelerators, proton therapy). However, all tools are intended for patients and thereby not suitable for the submillimetre dimensions of small animals. For this reason, *μ-RayStation 5* has been developed in collaboration with RaySearch. Based on a research version of the clinical TPS, functionality has been expanded to comply with the requirements of small animal irradiators. Import/export options are available for various image modalities. Furthermore, it provides versatile manual and automatic tools for contouring as well as different methods for rigid and deformable image registration. Plan design is supported for 3D conformal radiotherapy using fixed beams and static arcs including different features for plan evaluation and comparison. Dose calculation is performed by the VMC++ Monte-Carlo engine. Small animal irradiators can be modelled by specifying characteristics of design features and beam shaping components as well as dosimetric properties of the irradiation field. The respective machine models were validated by comparing calculations with radiochromic film (RF) measurements in homogeneous media as well as with an existing Monte Carlo GATEv7 model in heterogeneous media and a mouse CT.

Results: Tools for contouring and image registration as well as the dose grid have been modified to support dimensions down to 0.1 mm. Machine models include characteristics such as the geometry of cone-shaped beams (e.g. distances, aperture sizes), a focal spot model (e.g. 2D Gaussian distribution) and a photon energy spectrum of an X-ray tube generated from *SpekCalc*. *μ-RayStation 5* calculations and RF measurements agreed within 3 % and a maximal distance-to-agreement (DTA) of 0.2 mm at 50 % of the central dose. In heterogeneous media, the mean absolute error was below 2.2 % in each medium and DTA was 0.1 mm at interfaces. For the mouse CT, also good agreement was found.

Conclusion: *μ-RayStation 5* provides comprehensive functionality of a clinical TPS for small animal studies, allowing an efficient experimental workflow for experienced *RayStation* users. Both in-house and commercial systems were successfully modelled. Flexibility of the software facilitates adaption to other small animal irradiators and expansion of usage for preclinical research including proton irradiations.

Quantifying the setup uncertainty of a stereotactic murine system using the image guidance of the X-RAD SmART irradiator.

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Introduction: Developments in small animal irradiators are critical tools for translational research in radiation oncology. These modern irradiators offer numerous advantages over the previous generation of technologies, including image guidance systems, which provide the potential for increased accuracy in target localization, and advanced radiation delivery techniques, which may better model the paradigms utilized in clinical radiation treatments. However, with these advancements still comes a need to balance enhanced precision with the fundamental requirement for efficiency in high throughput animal experiments. This study seeks to investigate the reproducibility of murine cranial positioning using solely a stereotactic stage for mouse setup, and to quantify the potential improvements or margin reductions that could be gained from using the on-board image guidance of the X-RAD SmART irradiator (PXI, North Branford, CT).

Materials & Methods: Athymic nude mice (N=5) were anesthetized under isoflurane and cranially fixed on a stereotactic stage using a bite block and ear bars (Kopf, Tujunga, CA). Each mouse was imaged via orthogonal pairs of fluoroscopic images (fluoro) and cone-beam computed tomography (CBCT). For both imaging modalities, a target was placed at the midline vertex of the mouse skull, the stage shifted to that target, and the couch positions recorded. The mouse was then removed from the stage and this process was repeated twice more for each animal (thus, N=15 measurements). Since volumetric imaging is the gold standard for localization, the mean CBCT coordinates were used to define "stereotactic couch coordinates." From these stereotactic coordinates, both fluoro and CBCT positional shifts were calculated to quantify the setup accuracy based on couch coordinates alone.

Results: The mean vector shift between stereotactic setup and CBCT alignment was 0.63±0.31 mm. The mean shift between fluoro and CBCT alignments was 1.39±0.40 mm, which was dominated by the average longitudinal shift difference of 1.33 mm.

Conclusion: Preliminarily, fluoroscopy was not found to offer significant localization benefit beyond stereotactic positioning, nor was there significant increase in temporal efficiency versus CBCT. Using this methodology, we will develop class solutions for stereotactic coordinates based on mouse age/size; once derived, these stereotactic coordinate class solutions must be validated using an alternate specimen cohort. Subsequently, this data will confidently inform the margin required to ensure accurate dose coverage and will establish requirements for frequency and/or utilization of CBCT. Ongoing studies will evaluate the need for ear bars, which likely provide increased accuracy and repeatability, potentially at the cost of setup efficiency and increased plan complexity (due to avoidance requirements).

Previous glioblastoma irradiation experiments at our institution utilized a cesium irradiator with a whole head radiation approach, where oral cavity toxicities limited the deliverable dose to 20 Gray. These preliminary findings and established methodology will assist in the design of a mucosal sparing beam geometry, which could potentially allow for an increased radiation dose that better translates to the dose delivered to human patients

µIMRT on the SARRP using the motorised variable collimator

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Introduction: Bringing small animal irradiations closer to clinical radiotherapy is expected to result in more meaningful and translatable preclinical radiation research. However, the current standard in preclinical irradiations differs from clinical practice. In clinical radiotherapy, beam modulation for intensity modulated radiotherapy (IMRT) treatments is achieved with multi-leaf collimators. But due to the small size of the animals in preclinical radiation research and the resulting small beam sizes, this method is not easily downscaled. We developed a µIMRT framework to deliver intensitymodulated beams with two sets of orthogonal jaws, and present the technical implementation.

Materials&Methods: We perform our research on the Small Animal Radiation Research Platform (SARRP), which is equipped with a motorized variable collimator (MVC) and a magnetic shutter system. The MVC consist of two pairs of focused, orthogonal jaws, capable of delivering rectangular fields from 1x1mm2 to 80x40mm2.

To create the µIMRT plans, we developed a jaw-only direct aperture optimization based on [Wild et al, MedPhys 42(5), 2015] and implemented it in our in-house treatment planning system Dynaplan. Dose influence matrices are calculated with our in-house dose engine [Reinhart et al, BJR 90(1069), 2017]. The output factors are interpolated from a set of Gafchromic EBT3 film measurements for various rectangular shapes. Since the MVC only supports symmetric jaw movements, we achieve offaxis fields with automated couch shifts.

We deliver the µIMRT plans as step-and-shoot irradiations using the shutter system to interrupt the beam between segments. To this end, we developed our own control software for the SARRP. It controls the beam, couch, jaws and shutter system in a fully automated one push button solution. We present a first proof of concept on a cylindrical, mouse-sized solid water phantom with radius r=1cm and height h=7cm. We created multiple 7-beam µIMRT plans for an artificial, horseshoeshaped target (V=85mm3), to be irradiated with 8Gy, which wraps around a cylindrical critical structure (r=1.5mm). We report irradiation times, varying the number of apertures from 35 to 140. For evaluation purposes, we deliver the optimized fluence patterns to a solid water stack from a gantry angle of 0°**.** We recalculate the doses for this setup, and compare the calculations to Gafchromic EBT3 film measurements in 1cm depth.

Results: We successfully implemented a µIMRT framework on the SARRP. All optimised treatment plans accomplish high target coverage and sparing of the critical structure. Preliminary measurements of the individual fluence profiles with Gafchromic EBT3 films demonstrate the feasibility to deliver the intensity-modulated beams on a small animal scale. While we observe discrepancies between the measured and calculated doses of up to 2Gy, these occur at the beam edges due to the simplified head model and the limited accuracy of the collimator. We are currently fine-tuning the head model and improving the collimator. The delivery times range from 4.1min for 35 apertures to 11.3min for 140 apertures.

Conclusion: The proposed µIMRT framework will allow small animal irradiations similar to current clinical practice. The preliminary results are promising, and future work will focus on the rigorous commissioning of the system.

ESTRO ACROP: Technology for precision small animal radiotherapy research: Optimal use and challenges

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Precision (Image-Guided) Small Animal RadioTherapy (SmART) is a novel field of pre-clinical research, where the aim is to investigate the action of radiation, possibly in synergy with other agents, in small animal models for cancer and normal tissue. Both fundamental radiobiology and pre-clinical trials are the goal. The novelty of the field is in the very precise radiation beams that are applied to the animal models, possibly guided by high-resolution imaging and sophisticated treatment planning.

In the framework of ACROP (Advisory Committee in Radiation Oncology Practice, published by the European SocieTy for Radiotherapy and Oncology's) coordinating the issuing of guidelines, a writing committee established in 2016 had the mandate to review the current state of the art in this new field. The writing committee surveyed the current state of the art in advanced preclinical research, and recently issued recommendations:

-for commissioning and operating precision irradiators (standardize irradiation conditions, beam quality and dose reporting),
-for commissioning and operating pre-clinical imaging equipment (onboard CT and dual-energy CT imaging, bioluminescence imaging, and other forms of imaging such as MR, PET/SPECT, ultrasound), along with guidelines for image registration.

In addition to photon irradiation, special attention will be given to the emerging research field of pre-clinical precision image-guided irradiation using particle beams.

Recommendations will also be made on treatment planning, data handling and standardization, training and support.

The resulting recommendations issued by the writing committee, detailing how to use the available technology optimally to achieve best standard practice are published in Radiotherapy & Oncology 2017 on how to use the available technology optimally to achieve best standard practice. This is the first set of ACROP guidelines published by ESTRO on preclinical research.

Abstracts: Posters

Brain stereotactic radiotherapy damages recorded by Functional MRI.

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Introduction: Radiotherapy is commonly used in brain malignant diseases (i.e. metastases or Glioblastoma) and in benign diseases (i.e. meningioma, epilepsy, vestibular schwannoma or Parkinson disease). The use of stereotactic radiosurgery (SRS) allows high doses irradiation in a single fraction (e.g. 120Gy) within a small brain volume. After irradiation, morphological and functional cerebral changes occur depending on the total dose, the dose per fraction and the irradiated brain volume. The aim of this work is to use f-MRI to record the adult normal brain modification after irradiation and to identify new parameters of brain radio-damages.

Materials&Methods: To reproduce brain SRS, of a small part of adult brain mice (n=72) was irradiated with a 2mm-collimator (X-Rad SmART-PXI) and with dose schedules: 1X20Gy, 3X10Gy and 4X5Gy. A sham population of mice processed in the same manner as irradiated mice served as control. With a dedicated 9.4-T MRI (Agilent), we performed imaging of brain mice longitudinally. Imaging was realized once before RT as reference level and after RT every month for 5 months. For each mouse we acquired 14 slices of 1 mm thickness and 0.5 mm gap with an "in plane voxel resolution" of 0.5 mm. We performed T1-weighted, T2-weighted, T1-mapping, T2-mapping and DW-MRI. For DW-MRI, we performed Fast Spin Echo MultiSlice sequences, with 9 different B-value and B0 (from 20 to 1000). We performed IntraVoxel Incoherent Motion (IVIM) analysis to obtain information on intravascular diffusion, related to perfusion (*F*: perfusion factor).

Results: Changes were observed only in mice irradiated with 120Gy. Modifications were present in T1 and T2 anatomic images, in T1 mapping, ADC, D and F but no modifications were recorded in D* or T2 mapping. We don't observed modification during the first 4 weeks, at week 5 after SRS, the modification appears and then stabilized 7 weeks after irradiation. The mean values for the control group were stable during the 5 months (ADC 0,73µm^2/ms; D 0,66µm^2/ms; F 4,67%, T1 1,25 sec). For the 120Gy group, values were significantly higher after 5 weeks (Δ = compared to the control group) with ADC 1,66µm^2/ms (Δ=151%); D 1,37µm^2/ms (Δ=107%); F 18,84% (Δ=303%); T1 1,99 sec (Δ=59%). No specific behaviour changes were observed during all the experiment.

Conclusion: In this work, we studied normal brain modifications after SRS therapy with anatomical and functional MRI. SRS doses and schedules reflected those used in clinic for tumor treatment or functional SRS. We showed an increase of ADC value 5 weeks after one single dose of 120Gy, compared to normal brain tissue. These results are consistent with previous result published associated with radio-necrosis. In addition, we highlighted an increase of IVIM parameters D and F and an increase of T1 mapping in radio-necrosis area. These results increase the numbers of MRI parameters that could be used for following brain damage after radiation.

Initial Treatment Plan and Delivery Accuracy Assessment of the Small Animal Radiotherapy Research Platform

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Background: The Xstrahl Small Animal Radiation Research Platform (SARRP) is used to irradiate mice and rats, and in Glasgow it is used for oncological brain irradiation of murine subjects. In the interest of sparing normal tissues and increasing conformity of dose delivery to tumours, more complex treatment plans are investigated.

Aims/Objectives: Three different plan types were explored, reporting in terms of the dose delivered to a tumour and various organs at risk in an example mouse subject with an associated magnetic resonance scan of the brain. The choice of treatment plan will be dependent on the need to spare the contra-lateral hemisphere of the brain. The ability of the SARRP to deliver these plans was investigated by using the same plan parameters and planning on a phantom with Gafchromic EBT3 film placed at the isocentre, and comparing the delivered dose to the planned dose.

Methods/Results: Plans included static single beams, a single plane gantry arc and a couch rotation arc. A calibration curve for the delivered doses to the film was established and used to convert the delivered doses on the film into true dose maps. A 2D gamma analysis implementation was used for comparison. A summary of the best achievable gamma criteria are given, where the dose difference and distance to agreement are selected as 'best available' when they are the tightest tolerances where greater than 95% of pixels in the image pass the criteria.

Single beam plan: 3%/2mm or 2%/3mm (suggested error in set up of phantom)

Single plane gantry arc: 0.5%/2mm or 3%/1mm

Couch rotation arc: 0.5%/3mm or 1%/1mm

The sparing of various organs at risk show that single plane gantry arcs spare the contra-lateral brain more than the couch rotation arcs, at the expense of increased dose to the rest of the ipsi-lateral brain. Different tumour presentations will affect the choice of plan.

Conclusions: The SARRP is capable of delivering complex plans accurately. The choice of treatment plan will depend on the presentation of the tumour in the animal; the dose to volume analysis should help to aid these decisions.

Complete Response and slight side effect after IMRT for a feline unknown nasal tumor case

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Introduction: Feline nasal tumors are malignant and considered to be locally invasive. Lymphoma is the most common of nasal malignancies, followed by adenocarcinoma and squamous cell carcinoma. The patient in this report showed visible nose bulges, nasal discharge, epistaxis, sneezing and dyspnea. She accepted medical treatment for more than 2 months, without significant improvement. The biopsy examination failed to definite diagnose, but its aggressive biologic behavior presumably considered as a malignant tumor. Computed tomography images showed the left nasal cavity filled with lumps, the disappearance of the nasal septum, bone lysis, and the mass invaded into the orbit. Because of its local aggressive invasiveness and closeness to critical organs, we decided to use IMRT to minimize the damage to normal tissue.

Materials & Methods: The prescription dose was 40 Gy in 10 fractions on a Monday, Wednesday and Friday schedule. The patient was treated with photon beam delivered with 6MV linear accelerator. The planning target volume included a whole nasal cavity and adjacent lymph nodes.

Results: Clinical symptoms improved significantly after radiation therapy, and showed a complete response. A slight side effect was observed with alopecia, pigmentation, dermatitis, purulent nasal discharge and conjunctivitis.

Conclusion: Although the mass was undiagnosed, according to the local invasiveness of the mass and location, it is assumed to be a malignant tumor. We suggest hyperfraction regime with IMRT as the standard protocol, to minimize the radiation toxicity to achieve the better outcome.

Longitudinal and regional assessment of radiation-induced lung density changes using automated image registration in µCBCT mouse data

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Introduction: Sensitive, clinically relevant preclinical models are much needed to assess the effect of novel radiosensitizers and mitigators on radiation dose-limiting lung toxicity. In this work we explored whether µCBCT allowed such sensitive assessment of lung damage in a murine fibrosis model. Image registration was performed to automate the lung density quantification. Pathological analyses indicated regional differences in fibrosis that contributes to lung density changes. We therefore also compared the use of different regions of interest (ROI) for density measurements in our automated analysis.

Materials & Methods: The right lungs of 10-week-old C57Bl6/J mice were irradiated with single doses (0, 15, 18, 21, 24, 27 Gy) using the XRAD 225Cx µIGRT system. Heart and left lung were shielded by a custom-made collimator. µCBCT was used for accurate radiation beam positioning. At different time-points, baseline and post-irradiation (0, 8, 34, 37, 40 weeks), 5 mice of each group were µCBCT-scanned at 100kV. At 40 weeks, mice were sacrificed for histological and pathological assessments, which confirmed significant fibrosis levels in the right lung at > 18 Gy.

Right lung densities were quantified, semi- and fully- automatically, for each subject:

Semi-automatic: (i) a central slice in the coronal plane was selected; (ii) an area that would exclude larger blood vessels and bronchi was manually delineated.

Fully-automatic: (i) all time-points were registered to the respective baseline scan at week 0; (ii) the baseline scan was registered to a template image; (iii) the ROIs delineated on the template image were propagated to all subsequent time-points.

Six sets of template lung ROIs were tested that varied in size (1–33 mm^3) and location.

Results: After radiation, µCBCT-based density values increased over time in a dose-dependent manner, maximizing by 37 weeks and later. A clear radiation dose-dependence was found in the right lung densities from 34 weeks on. This was the case for five out of six sets of ROIs in the fully automated assessments. Density changes are most rapid after irradiation with higher doses and range from 9-50 HU/week in the last weeks. The semi-automatic quantification of main and lobar bronchi-free areas showed a similar radiation dose response. Maximal dose-response density slopes increased with time and were found to differ between the six ROI sets (20-40 HU/Gy). The sensitivity to depict damage also depends on the variation within a treatment group of mice. Assuming a fairly strict, but clinically relevant, radiation dose modifying factor (DMF) of about 1.2 (i.e., caused by an experimental radiosensitizing drug), we next tested whether it was possible to depict such toxicity risks sensitively. Albeit the small group size, we found a significant density change in several of the fully automated quantifications at 37 and 40 weeks, due to a 3 Gy dose increment.

Conclusion: µCBCT analysis in murine lung fibrosis models enables the sensitive detection of potential radiation response modulators. Fully automated registration and image density acquisition is feasible and satisfactorily sensitive and therefore supports large scale studies.

Dose painting by combined couch motion and irradiation on an image guided small animal radiotherapy platform

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Introduction: Hardware for precision image guided small animal radiotherapy is evolving and catching up with clinical irradiation capabilities. Improvements of preclinical radiotherapy platforms require improving software to plan, evaluate and control the hardware for careful treatment delivery. Treatment goals and restrictions in preclinical research can be vastly different clinical therapy. Therefore, preclinical research platforms should not necessarily pursue the same development paths of their clinical counterparts. The purpose of this study is to improve treatment delivery by adding specimen couch motion during treatment delivery as a degree of freedom.

Materials & Methods: For small animal treatment planning we used a research version of the Monte Carlo based treatment planning system SmART-ATP. SmART-ATP was extended with a module for beam on time optimization that uses an optimizer to determine Pareto-optimal solutions using user provided constraints and weights for objectives. SmART-ATP was also extended to enable calculation and handling of large numbers of radiation beams, to subsequently use those data as input during plan creation and optimization. Further extension was performed to generate beam configurations with many control points to enable couch motion during irradiation, and thereby mimic dose painting. Treatment plans were delivered using a Precision X-Ray XRAD 225Cx, assessed using radiochromic film, and compared to calculated dose distributions.

Results: SmART-ATP was successfully extended with modules to perform large numbers of dose calculations and beam on time optimization. The XRAD 225Cx proved to be able to accurately deliver treatment plans with beams consisting of several hundred control points. Thereby, the XRAD 225Cx was able to irradiate larger areas using a dose painting strategy. A disadvantage of applying this strategy proved to be less sharp dose gradients at the edge of target volumes. This trade-off between sharper dose gradients using a lower dose rate, and more gradual dose gradients at a higher dose rate, can be made on a case by case basis and the availability of different beam sizes.

Conclusion: To maximize the benefits of image guided small animal radiotherapy, treatment planning automation can help to achieve the delivery of more complex dose distributions by using treatment plans with many control points. Specimen couch motion during irradiation can then be applied to deliver more conformal dose delivery, and heterogeneous dose distributions.