Submitted abstracts for talks at Cancerfondens Planeringsgrupp för Onkologisk Radionuklidterapi höstmöte 2017

Lovisa Lundholm *Stockholms universitet*

The role of chromatin in response to alpha and gamma radiation in breast cancer cells

Chromatin structure is a factor in the sensitivity of cells to gamma radiation, but it has not been thoroughly studied in response to high linear energy transfer (LET) radiation such as alpha radiation. The focus of this study was to evaluate the relative importance of DNA damage induction and repair for sparsely ionizing gamma radiation which mainly damages the DNA by induction of free radicals, where compact chromatin protects the DNA, compared to densely ionizing alpha radiation where free radicals play a minor role due to its direct interaction with DNA and compact chromatin mainly would inhibit DNA repair. An opening of chromatin using 0.5 and 1 µM of the histone deacetylase inhibitor trichostatin A (TSA) was confirmed by an increase in the acetylated lysine 8 of histone H4 (H4K8ac). Pretreatment with TSA for 18 h produced opposite results for the different radiation types in the aggressive, triple negative breast cancer cell line MDA-MB-231. TSA pretreatment before gamma radiation resulted in reduced clonogenic survival, while for alpha radiation it caused increased survival. Gamma H2AX foci were analysed from 15 min up to 24 h after exposure, and displayed a clear increase post gamma radiation upon TSA pretreatment, which is in line with an increased damage induction. For alpha radiation on the other hand, after a peak at 15 min, the number of foci was lower or similar using TSA pretreatment, which is in line with an increased repair. In addition, a compact chromatin has been linked to a poor prognosis, here we show that the basal level of the heterochromatin markers trimethylated lysine 9 of histone 3 (H3K9me3) was higher in the MDA-MB-231 cell line which is more resistant towards gamma radiation compared to the hormone receptor positive MCF7 cell line. In conclusion, these data indicate a higher relative importance of DNA damage induction post gamma radiation, while a delayed but still more efficient repair is suggested as the net effect post alpha radiation in the state of opened chromatin.

Britta Langen *Göteborgs universitet*

Age and sex: important variables in biomarker discovery for radiotherapy and risk estimation

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Background: Gene expression profiling is a promising tool for absorbed dose estimation in the clinical setting or in a radiation hazard event. Despite growing awareness, age and sex are often neglected as intrinsic variables in pre-clinical studies. If not controlled for, age and sex can lead to bias in the dose-response of biomarker candidates and consequently misrepresent absorbed dose in clinical practice or when screening (potential) radiation hazard victims.

Aim: To assess the bias from age and sex in the dose-response of transcriptomic and proteomic regulation. In addition, sex bias was assessed for two commonly used mouse strains.

Methods: Mice (n=4–5/group) were irradiated with 0.5 Gy absorbed dose from 15 MV (nominal) photon beams in a whole-body irradiation setup. Groups consisted of male and female C57BL/6N mice (7 and 18 weeks old for both sexes), and male and female BALB/c nude mice (7 weeks old). Ages were chosen to represent juvenile and adult individuals. Matching control groups for sex, age, and strain were mock-treated. Mice were killed 24 h after treatment and spleen, whole blood and blood plasma samples were snap-frozen in liquid nitrogen. Subsequently, samples were processed and subjected to transcriptomic (spleen and whole blood; RNA-seq) and proteomic (spleen and plasma; LC-MS/MS) analysis.

Results: Preliminary data indicates that the sensitivity and specificity of biomarkers for low-dose radiation exposure can depend on age, sex, and strain of the chosen mouse model, while the extent of differences between these variables appears to be tissue-specific. Functional pathway analysis is ongoing in order to assess how age- or sex-differences may relate to different health risks from low-dose exposure.

Conclusion: Sex and age can create severe bias in biomarker screening. This work contributes to the identification of accurate biomarkers for radiation exposure that are sex- and age-specific for the exposed individual.

Bertil Persson *Lunds universitet*

The synergy between ionizing radiation and immunotherapy in the treatment of prostate cancer

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Already at the start of the millennium we were exploring the therapeutic effect of radiation therapy (RT) combined with immunization by syngeneic Interferon-gamma secreting tumour cells on N29 and N32 brain tumours (Persson et al., 2002). Already the results of 8 Fischer 344 rats in each group with N29 tumour implanted in the brain, indicated that a single-fraction radiation therapy with 5 Gy combined with immunization increased the survival time significantly by 87% compared to the controls, and resulted in 75% complete remissions (Persson et al., 2010). Similar results in various pre-clinical tumour models were soon found by other researchers:, Lumniczky Safrani 2002 in Hungary, Prins and Graf 2002, Sandra De Maria 2005, Newcomb 2006, Sharp NCI 2007 all in the US. In 2012 Hannan et al found that a single fraction external beam RT of 10 Gy combined with Listeria PSA-vaccine (ADXS31-142) caused significant tumour regression by augmenting PSA-specific immune response and claimed it could serve as a potential treatment regimen for prostate cancer (Hannan et al., 2012). From May 26, 2009, to Feb 15, 2012, a phase 3 trial, CA184-043, evaluated overall survival with radiotherapy (RT) followed by immune therapy or placebo in 799 patients with advanced metastatic castration-resistant prostate cancer (mCRPC) (Kwon et al., 2014). But instead of vaccine for immunization, they used anti-CTLA-4 (Ipilimumab). The patients were randomized to receive a single fraction of 8 Gy RT to bone metastases, followed by either Ipilimumab (N = 399) or Placebo (N = 400). After 3 years

of evaluation they found a significant increase in the overall survival in the group treated with RT and immune-therapy. In addition, subgroup analyses suggest that patients with lower disease burden may be more likely to benefit from the combined treatment (Fizazi et al., 2014). Encouraged by these results we will proceed in our efforts of to elucidate the preferred immunotherapy, and the timing, fractions, absorbed dose, and volume of RT required to maximize the effects of combination immunotherapy and RT.

Renata Madru *Lunds universitet*

Simultaneous preclinical PET-MRI study of lymphatic drainage of chelator free ^{64}Cu-labeled nanoparticles

Hybrid PET-MRI systems have been taken in use as new clinical diagnostic tools including detection and therapy planning of cancer. To reduce the amount of contrast agents injected in patients while fully benefit of both modalities, dual-modality probes are required. This study was aimed first, to develop a hybrid PET-MRI probe by labeling superparamagnetic iron oxide nanoparticles (SPION's) with 64Cu using a fast and chelator free conjugation method, and secondly, to demonstrate the ability of the agent to target sentinel lymph nodes (SLN's) in vivo using simultaneous PET-MRI imaging. High labeling efficiency of 97% within 10-15 minutes was demonstrated at room temperature. ^{64}Cu-SPIONs were chemically stable in mouse serum for 24 h and after intradermal injection in the hind paw of C56 mice, demonstrated specific accumulation in the SLN. Both modalities clearly could visualize ^{64}Cu-SPIONs, using dynamic and static images up to 24 h. The use of a single hybrid probe and simultaneous hybrid imaging provides an efficient, complementary integration of quantitation and is expected to improve pre-operative planning and intra-operative guidance of cancer treatments. The contrast enhancement in images is concentration and time dependent, and therefore the amount of injected probe and imaging time point must be chosen systematically.

Emma Wikberg *Sahlgrenska universitetssjukhuset*

Comparison of an analysis method and visual assessment concerning liver metastases in ^{111}In-octreotide SPECT imaging including Monte Carlo based reconstruction

Aim: This work aims to increase the diagnostic accuracy of SPECT imaging in ^{111}In-octeotide diagnosis. This is done by improving image quality through implementation of Monte Carlo (MC) based OSEM reconstruction and by application of an analysis technique called nNUFTI.

Materials and methods: The nNUFTI method, developed at Sahlgrenska University Hospital, is a homogeneity measure and a statistical approach that assumes a different uptake foci distribution in healthy livers compared to livers with tumor involvement. The normalized number of uptake foci is plotted against a threshold index (ThI) and the quantitative measure used is ThI at 25% of maximum uptake foci. Tumour involvement will shift the curves towards higher ThI. We have previously shown that in a group of 53 randomly selected ^{111}In-octreotide tumour negative patients (iterative reconstruction with attenuation correction, IRAC OSEM) nNUFTI was able to separate the group of patients (n=40) that stayed healthy in a three-year follow-up from the group of patients (n=13) that had emergent liver metastases (p<0,01). 5 out of these 13 patients were clearly above the normal group in ThI. In the present study, the patient material has been reconstructed using Sahlgrenska Academy Reconstruction Code (SARec) in order to improve image quality. SARec is a reconstruction method where MC simulations are used in the forward projection of an iterative

OSEM reconstruction. An observer (a trained nuclear medicine specialist) has reviewed 15 patients (of which 9 had emergent liver metastases) once again using reconstruction with IRAC OSEM and using SARec. Criteria evaluated were liver metastases yes (1) or no (0) with confidence level 1-3 (1-low/uncertain, 2-moderate/probable and 3-high/certain). The observer knew there were patients with emergent liver metastases in the follow-up.

Results: The observer could not detect any liver metastases in any of the patients, neither using IRAC OSEM nor using SARec. Confidence level was lower for SARec than for IRAC OSEM.

Conclusion: Although biased (the observer was aware that there were patients with liver metastases in the follow-up) the observer could not detect any liver metastases. SARec did not change the visual outcome but lowered the observer's confidence level concerning lack of metastases. This shows the importance of and need for a quantitative measure like nNUFTI. Future plans include nNUFTI analysis using SARec and improvement of the visual quality of SARec images to increase confidence levels.

Daniel Roth *Lunds Universitet*

A method for ^{177}Lu-PRRT tumour dosimetry based on hybrid planar-SPECT/CT images and semi-automatic segmentation

Aim: As part of an ongoing ^{177}Lu-Dotatate clinical trial (www.clinicaltrials.gov, NCT01456078) patient image acquisitions are performed for each cycle, and consists of a combination of anterior-posterior whole-body scans at four time points and one SPECT/CT. Image-based activity quantification is made as part of this clinical trial and methods have been presented previously. The aim of this work is to develop and evaluate a hybrid planar/SPECT-based method for tumour dosimetry.

Materials and methods: An active ray-based segmentation method is developed and used for tumour delineation in the planar images. SPECT image segmentation is performed using a method based on deformable surfaces. Hybrid planar-SPECT/CT time-activity concentration curves (TACCs) are calculated, where the curve shape is derived from the planar images and is then scaled to the activity concentration estimated from the SPECT image. Evaluation is performed in six patients for whom parallel acquisitions of 4 whole-body scans and 4 SPECT/CT have been made. In addition, Monte Carlo simulated images are used, emulating ^{177}Lu-Dotatate pharmacokinetics and the imaging protocol employed. The hybrid method is evaluated in terms of effective half-lives, time-integrated activity concentration coefficients and mean tumour absorbed doses, using as reference either the SPECT-derived TACCs for patients, or the reference values for the simulated images. The operator dependency in the planar segmentation is also evaluated by comparing regions of interest and effective half-lives obtained from delineation by two operators.

Results and discussion: In the patient material, the estimated absorbed doses of the hybrid method are on average 6 % below those of the SPECT-only method, with a standard deviation of 8 %. In the simulated images, it is seen that small tumours are unsuitable for evaluation due to a greater sensitivity to volume inaccuracies and partial volume effects causing activity concentration underestimations. Tumours located in a surrounding with high peritumoral activity in the anterior-posterior direction may be less suitable for evaluation since the TACC shape and effective half-life can be affected by the peritumoral pharmacokinetics. The operators' results generally agree well, with a median Dice similarity coefficient of 0.97 and a mean absolute half-life difference of 2 %.

Conclusions: The hybrid planar-SPECT/CT tumour dosimetry method performs well in relatively large tumours not surrounded by high-activity uptakes in the anterior-posterior direction. The planar semi-automatic segmentation offers less time-consuming delineations compared to manual segmentation with little operator dependency.

Tom Bäck *Göteborgs universitet*

High-resolution Alpha Camera imaging as a tool for developing Targeted Alpha Therapy

Targeted alpha therapy (TAT) attracts increased interest, particularly for treatment of metastatic cancer. Before its full clinical potential can be realized, however, many of the current gaps of knowledge must be overcome. The often missing information relates to the highly localized, and often very heterogeneous, alpha-particle radiation energy distribution. The concept of mean absorbed dose to whole organs will therefore in many cases be misleading when evaluating, for example, dose-response relationships. Instead, the dosimetry from alpha-particle irradiation must be derived for the small-scale (sub-organ) level. This can be achieved using small-scale imaging of alpha-particle decay and dosimetric modelling. The alpha camera imaging system was developed to provide high-resolution quantitative imaging of alpha particles in tissue. The key elements of this system have been previously published. Here, an updated overview of the alpha camera imaging platform and results from a wide range of collaborative and published studies will be provided. Distribution data will be presented from imaging of several alpha-emitters including Ra-223, Th-227, Bi-213 and At-211. Several different issues of TAT will be addressed. Examples of such issues include intra-tumoral and intra-renal distribution; PRIT vs RIT; uniform vs non-uniform uptake in normal organs; and bone marrow imaging. Illustrative examples will be presented of how alpha camera imaging can be used for small scale 3D- dosimetry using voxel dose-kernel computations. The capability of quantum detection and imaging, i.e. single-event imaging of individual alpha particles will also be shown. Importantly, preliminary imaging results will be presented for the first two patients of an ongoing bone biopsy study on patients being treated with Ra-223 chloride for treatment of castration resistant metastatic prostate cancer. This biopsy study aims to derive small-scale experimental data on the Ra-223 uptake profile and distribution in skeletal tumor lesions in the bone and marrow compartments.

Anja Mortensen *Uppsala universitet*

Preclinical evaluation of a novel engineered recombinant human anti-CD44v6 antibody for potential use in radio-immunotherapy

CD44v6 is overexpressed in a variety of cancers, whereas in normal tissue it is only expressed in two subtypes of epithelium, making it a promising target for radio-immunotherapy (RIT). In this study, we have developed a novel engineered recombinant monoclonal anti-CD44v6 antibody, AbN44, and assessed its potential for use in RIT using either ^{177}Lu or ^{131}I. In vitro affinity and specificity assays characterized the binding of the antibody labeled with either ^{177}Lu, ^{125}I or ^{131}I. The therapeutic effects of ^{177}Lu-AbN44 and ^{131}I-AbN44 were investigated using in vitro 3D tumor models with varying CD44v6-expression. Finally, normal tissue biodistribution and dosimetry was assessed in vivo for ^{177}Lu-AbN44 and ^{125}I-AbN44/^{131}I-AbN44 using a mouse model. All AbN44 radioconjugates bound with high specificity to CD44v6 in vitro. In the in vitro 3D tumor models, dose dependent therapeutic effects were seen with both 1^{77}Lu-AbN44 and ^{131}I-AbN44, with a greater significant therapeutic effect observed on cells with higher CD44v6 expression. Biodistribution studies demonstrated greater uptake in liver, spleen and bone of ^{177}Lu-AbN44, and longer circulation in the bloodstream of ^{125}I-AbN44. In dosimetric calculations, critical organs for ^{177}Lu-AbN44 were liver and spleen, while the kidney and red marrow were considered critical organs for ^{131}I-AbN44. The effective dose was in the order of 0.1 mSv/MBq for both labels. We conclude that AbN44 binds with high affinity to CD44v6, and in vitro RIT demonstrated growth inhibition in a CD44v6-specific activity-dependent manner, regardless of radioconjugate, demonstrating the that both ^{177}Lu-AbN44 and ^{131}I-AbN44 are promising candidates. Furthermore, biodistribution and dosimetry studies of the conjugates demonstrated that both conjugates could be feasible for RIT. The specific therapeutic effects seen with radiolabeled AbN44 in the 3D tumor models in vitro, combined with the beneficial dosimetry in vivo, makes AbN44 a promising candidate for RIT.

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Andrzej Wojcik *Stockholms universitet*

DNA damage and repair after a combined exposure to alpha particles and X-rays

Under many situations, people are exposed to mixture of high linear energy transfer (LET) and low LET ionizing radiation (IR). To estimate the biological effect induced by combined IR is important for human radiation protection. Previous studies showed both additive and synergistic effect of combined IR of different LET and the outcome seemed to be dependent on the experiment setup. Earlier studies from our lab indicated a synergistic effect in cells exposed to mixed beams of alpha particles (high LET) and X-rays (low LET). But the mechanism of synergism is unclear until now. The purpose of our present research is to analyze the effect of combined action of alpha particles and X-rays on DNA damage and repair, and DNA repair response protein activation, in order to more closely investigate the mechanism of the observed synergism.

A dedicated mixed-beam exposure facility used in the experiments is installed and characterized at the Stockholm University, which allows simultaneously exposure of cells to 241Am alpha particles and X-rays under controlled temperature conditions. Experiments were done with human peripheral blood mononuclear cells (PBMC), where we previously saw synergism at the level of cytogenetic damage. Initial level of DNA damage and the kinetics of DNA repair were analyzed at the level of individual cells with standard alkaline comet assay, which can measure the total DNA damage of single stand breaks (SSBs), double strand breaks (DSBs), incomplete excision repair sites, cross links and alkali labile sites. The activation of proteins involved in the DNA repair after exposure, including phosphorylated p53 (S15), pATM (S1981), and pDNA-PKcs (S2056), were tested by western blot.

Both the initial level of DNA damage and DNA repair kinetics indicated a synergistic effect of mixed beams detected by comet assay. After 1 h incubation following 2 Gy of different exposures (X-ray, alpha particles and mixed beams), mixed beam caused the highest expression of phosphorylated p53 (S15) compared to X-rays ($p < 0.01$) and alpha particles ($p < 0.05$) and also highest expression of pATM (S1981) compared to X-rays ($p < 0.05$) and alpha particles ($p < 0.07$), which indicate a synergistic effect. But the expression of pDNA-PKcs (S2056) showed an additive effect. At 3h post-radiation, all of the three phosphorylated proteins showed a synergistic effect of mixed beams, although the increasing level of pATM induced by mixed beams was not significantly higher than X-ray and alpha particles. The level of pKu70 (S5) 3h post-radiation was tested by western blot too. With a high expression level in control, the level of pKu70 showed gradient increase after exposure of 2 Gy x-ray, alpha particles and mixed beams respectively.

Mixed beams might induce not only more initial total DNA damage, but also higher level of damage complex, compared to single type of radiation, in order to trigger stronger signal in p53 pathway. The results imply an interaction between the actions of high and low LET radiation. As the result suggests a synergistic effect of mixed beam, the cancer risk of exposure to mixed beams in radiation oncology may thus be higher than expected based on the additive action of the individual dose components.

Jens Hemmingsson *Göteborgs universitet*

Autoradiography and biopsy measurements of a resected hepatocellular carcinoma treated with ^{90}Y radioembolization demonstrates large absorbed dose heterogeneities

Radioembolization is an alternative treatment of hepatocellular carcinoma. This study has aimed at examining uptake differences between tumor tissue phenotypes and presenting a cross section of the absorbed dose throughout a specimen of liver tissue.

Methods: A patient with hepatocellular carcinoma was treated with ^{90}Y radioembolization whereafter liver tissue was resected. Gamma camera images and autoradiographies were collected and biopsies were measured and analyzed using light microscopy.

Results: Overall, 25 punched biopsies were measured and analyzed and four tissue regions were identified; normal tissue, viable tumor tissue with and without infarcted areas and tumor areas with post necrotic scar tissue. However, the autoradiography and biopsy measurements showed large dose differences between viable and post necrotic tumor tissue; 159 vs. 23 Gy.

Conclusion: In treating unifocal hepatic malignancies, ^{90}Y radioembolization produce absorbed dose distributions that are heterogeneous and could not be accurately determined with present gamma camera image techniques.