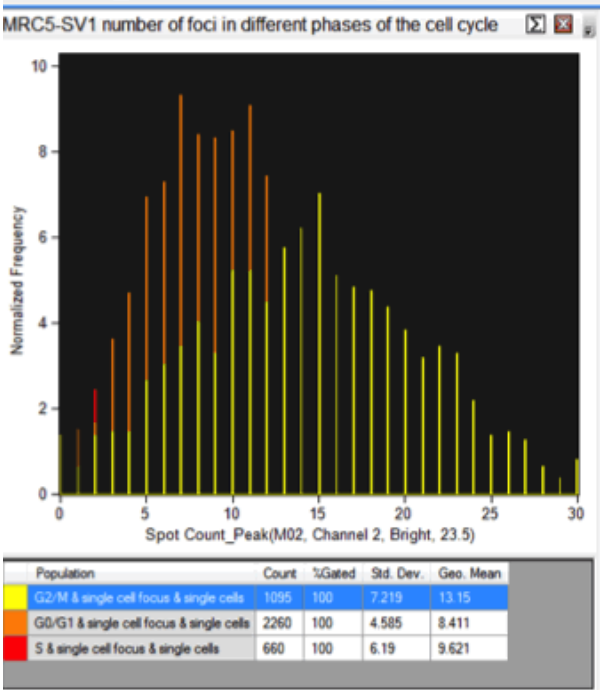
study and the outcome will inform future studies using γ-H2AX staining.

Material and Methods:
Fibroblast Cell Lines (SV40 immortalised)
– MRC5-SV1 - Repair normal.
– AT5BIVA – Classical ataxia telangiectasia.
Irradiation Cells
Irradiated with 2 Gy gamma radiation; harvested and fixed in 50:50 V:V methanol acetone. Time points: Un-irradiated, 30 min, 3, 5 and 24 hrs post irradiation.
Immunocytochemistry
Primary antibody: Anti-phospho-histone H2AX (Ser139), mouse monoclonal antibody clone JBW301 (1/10,000, Millipore).
Secondary antibody: Rabbit anti-mouse AlexaFluor488 (1/1000, Invitrogen).
DNA counterstained with Draq 5 (Biostatus Ltd.)
Imaging flow cytometry
Images of 5-10,000 cells captured

Results:



Population	Count	%Gated	Std. Dev.	Geo. Mean
G2/M & single cell focus & single cells	1095	100	7.219	13.15
G0/G1 & single cell focus & single cells	2260	100	4.585	8.411
S & single cell focus & single cells	660	100	6.19	9.621

Statistical Analysis • 30 minute time point, comparing mean foci count for G0/G1, S and G2/M with one-way ANOVA test:
– MRC5-SV1 (repair normal); $F(4,4010)=163.5$, $p < 0.001$
AT5BIVA (DNA repair defective); $F(2,2919)=421.3$, $p < 0.001$

Conclusion: We have identified cells in different phases of the cell cycle by analysing intensity of the Draq 5 nuclear stain and negating the need for extra staining. These data have shown a statistically significant difference between foci numbers in different phases of the cell cycle at one time point for a normal cell line and a DNA repair deficient cell line. Further work will look at differences in the cell cycle distribution between the two cell lines

Electronic Poster: Radiobiology track: Radiobiology of protons and heavy ions

EP-2071
Mitophagy and Apoptosis: mitochondrial responses to carbon ion radiation in tumor cells
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Purpose or Objective: Although mitochondria are known to play an important role in radiation-induced cellular damage response, the mechanisms by which tumor cells respond to the mitochondrial damage induced by high linear energy transfer (LET) radiation are largely unknown.

Material and Methods: Human cervical cancer cell line HeLa and human breast cancer cell lines MCF-7 and MDA-MB-231 were irradiated with high linear energy transfer (LET) carbon ions at low and high doses. Mitochondrial functions, dynamics, mitophagy, intrinsic apoptosis and total apoptosis, and survival fraction were investigated after irradiation.

Results: Compared with unirradiated cells, carbon ion irradiation resulted in the loss of mitochondrial membrane potential and fragmentation, suggesting mitochondrial damage was induced. Mitophagy and intrinsic apoptosis of tumor cells were the major responses to the carbon ion radiation induced mitochondrial damage. After exposure to low doses of carbon ions, cells initiated mitophagy to keep viability while tending to death via apoptosis at high doses.

Conclusion: Tumor cells through mitophagy and apoptosis respond to the mitochondrial damage caused by high-LET radiation according to the radiation dose. A threshold model depicting the fate of irradiated cells could provide a mechanistic explanation for differential mitochondrial damage response to high-LET radiation at low and high doses. Our data shed new light on understanding the mechanisms underlying high-LET radiation induced cell death.

EP-2072
Spatiotemporal dynamics of DNA damage in cells exposed to mixed beams of ionising radiation
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Purpose or Objective: A particular problem of modern external beam radiotherapy like IMRT and proton therapy is exposure of patients to scattered neutrons with a relative biological effectiveness (RBE) higher than X-rays. The interesting question is if there is an additive or synergistic effect of high and low linear energy transfer (LET) radiations when given together. If they act additively, then the risk of cancer can be deduced from the results of exposure to the single agents. Otherwise, RBE values must be generated for the mixed exposure scenarios or corrected to account for the synergism.

Material and Methods: The goal of this study was to analyse the kinetics of formation and repair of ionising radiation-induced foci (IRIF) in cells exposed to alpha particles, X-rays and a mixed beam of both radiations. To this end human cells were transfected with plasmids coding for the DNA repair the protein 53BP1 that are tagged with the green fluorescent protein (GFP). Cells were exposed to mixed beams in a dedicated exposure facility built at Stockholm University (SU). The facility is composed of a 50 MBq Am-241 alpha source and an YXLON 200 X-rays source. The alpha source is mounted on an inversed plate in a custom-designed irradiator which is kept inside a 37°C cell incubator.

Results: Spatiotemporal dynamics of 53BP1 foci formation and repair were recorded by time-lapse photography and image analysis. The distributions of cell frequencies with the specific size of foci and the size of foci itself were analysed. Moreover, Monte Carlo simulations (the PARTRAC code) were used not only for calculating radiation hits, but also for the biological damage in the DNA in terms of single and double strand breaks.

Conclusion: Exposure to a mixed beam induces complex DNA damage above the level expected from the additive action of

both radiations. Clustered DNA damage poses serious problems for the DNA repair and error-prone repair of DNA damage is associated with cancer induction. Increased damage complexity following exposure to mixed beams will suggest a higher than expected risk of cancer induction in modern radiotherapy. The results are consistent with the previous studies carried out at SU with different cell types and different biological assays. A synergistic interaction of the beam components was observed at the level of micronuclei, gammaH2AX foci and chromosomal aberrations.

EP-2073

Angio/lymphangiogenic, inflammatory and immune responses in head and neck cancer: proton vs photon

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Purpose or Objective: Due to its higher precision in tumor targeting, proton therapy could become the treatment of choice for head and neck cancer (HNC). Recent studies have shown that proton irradiation suppresses angiogenic genes and impairs tumor cell invasion/growth. According to the type of radiation, dose and fractionation, the objective of our study was to investigate the effect of proton (P+) versus photon (X) irradiations in squamous cells carcinoma (SCC), in respect of their proliferation, genes expression and proteins secretion involved in proliferation, angio/lymphangenesis, metastasis and anti-tumor immunity.

Material and Methods: Human SCC CAL33 cells were irradiated 1 to 3 times and evaluated on their proliferation (Cell counting), genes expression (qPCR) for proliferation (TRF2, PLK1), angio/lymphangiogenic (VEGF-A, VEGF-C, VEGF-D) inflammatory (IL6, IL8, CCL2, CXCL12) and immune (PD-L1) responses.and protein synthesis (ELISA).

Results: Cell proliferation was evaluated at 48h and at 3 weeks after 1 irradiation and showed a significant decreased in both X and P+, as compared to control but more important in P+. After 3 irradiations, cell proliferation at 48h was reversed and more decreased in X vs P+. Genes expression was investigated at 48h after 1 and 3 irradiations at 2 and 8 Gy. After 1 irradiation, the prevalence of gene expression levels associated with a poor outcome was higher in X than P+ at 8 Gy. After 3 irradiations, genes expression was increased for all but more important for P+ at 8 Gy. The highest expression was noted for VEGF-C (2 to 10 fold increase). The most frequent overexpression was noted for PD-L1. VEGF-C protein induction 48h after 1 and 3 irradiations was increased in both X and P+ groups but decreased in high dose P+, as compared to X.

Conclusion: Cell proliferation activity is in favor of P+ after a single irradiation, and X after multiple irradiations. Genes expression are overall increased in both X and P+, in a dose and fraction dependent manner, implicated in proliferation (TRF2), angio/lymphangiogenic (VEGF-A, VEGF-C, VEGF-D) and immune (PD-L1) responses. VEGF-C protein induction is increased after both X and P+ single and multiple irradiations, but in favor of P+, suggesting a lower lymphangiogenesis/metastatic dissemination immediately after P+. Our study sets the molecular basis for novel therapeutic approaches applicable to HNC in combination with X or P+ radiotherapy, such as angio/lymphangenic inhibitors or immune therapy as anti-PD1 or anti-PD-L1.

Electronic Poster: RTT track: Strategies for treatment planning

EP-2074

The comparison of properties for radiotherapy with flattening filter-free and flattening filter beam

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Purpose or Objective: The aim of this study was to appraise multiple properties for radiation therapy techniques applying flattening filter-free (3F) and flattening filter (2F) beam to the radiation therapy.

Material and Methods: Alderson rando phantom was scanned for computed tomography images. Treatment plans for intensity modulated radiation therapy (IMRT), volumetric modulated arc therapy (VMAT) and stereotactic body radiation therapy(SBRT) with 3F and 2F beam were designed for prostate cancer. To evaluate the differences between the 3F and 2F beam, total monitor units (MUs), beam on time (BOT) and gantry rotation time (GRT) were evaluated and measured with TrueBeam™ STx and Surveillance And Measurement (SAM) 940 detector was used for photoneutron emitted by using 3F and 2F.

Results: In using 3F beam, total MUs in IMRT plan increased the highest up to 34.0% and in the test of BOT and GRT, the values in SBRT plan by 3F beam decreased the lowest 39.8, 38.6% respectively. The values of photoneutron occurrence in SBRT plan using 3F beam decreased the lowest 48.1%.

Conclusion: According as the results, total MUs increased by using 3F beam than 2F beam in all treatment plans but BOT, GRT and photoneutron decreased by using 3F beam. From above the results, using 3F beam can have an effect on decreasing intra-fraction setup error and risk of radiation-induced secondary malignancy.

EP-2075

Evaluation of conventional versus IMRT based Prophylactic Cranial Irradiation treatment planning

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Purpose or Objective: Patients with Small-Cell Lung Cancer (SCLC) have a high risk of developing brain metastasis. Prophylactic Cranial Irradiation (PCI), is applied to SCLC patients that response to chemotherapy. It is well known that PCI is associated with an increase in median overall survival. There are approximately 84 incidences per year in central region DK. Radiotherapy (RT) to this group of patients is conventionally performed using opposed MLC defined static fields. However, treatment planning can be time consuming. The aim of this study is to evaluate time-effectiveness, by changing the treatment technique from conventional to IMRT based treatment planning of PCI patients.

Material and Methods: This retrospective study included twenty SCLC patients, all treated with conventional planned PCI. Each patient received 25 Gray in 10 fractions. An IMRT template was made (Eclipse Version 11.0, Varian Medical Systems, Palo Alto, CA) and for each patient an IMRT plan was generated by one IMRT optimization. One intermediate dose calculation was performed during optimization before the final dose calculation. The contoured structures used for comparison between IMRT and conventional planning were: ITV, PTV and left/right lens. The plans were evaluated and compared on; max- and minimum doses, the mean/maximum doses to the lenses, and the homogeneity index (HI). The HI was defined by D5%/D95%. Quality assurance of the IMRT plans was performed by recording Portal Dosimetry Images