Results: First, we identified that specific inhibition of PI4K αIIα using RNAi increased radiosensitivity in the human cancer cell lines we tested. In contrast, inhibition of other isotypes did not affect a radiosensitivity of these cancer cell lines. Next, in vitro kinase assays showed, simprevir, a selected anti-HCV agent via IC50 assay, inhibited activity of PI4K αIIα in a dose-response manner. Pretreatment of simprevir induced discernible downregulation of p-PKC and p-Akt and also increased clonogenic survival of UZS1, BT474, and HepG2 cells in vitro and also significantly delayed growth of mouse tumor xenografts in vivo. Simprevir caused prolongation of γH2AX foci after irradiation, decreased invasion / migration and downregulation of PD-L1 expression.

Conclusion: Targeting PI4K αIIα using anti-HCV agent could be a viable drug repositioning approach to enhance the therapeutic efficacy of radiotherapy for breast cancer, glioblastoma and hepatoma. (Work supported by grant #2013R1A1A2074531 from the Ministry of Science, ICT & Future Planning to Kim IA)

PV-0427
Real-time tumour oxygenation changes following a single high dose radiotherapy in mouse lung cancers


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Purpose or Objective: To investigate serial changes of tumor hypoxia in response to a single high dose irradiation by various clinical and pre-clinical methods in order to propose an optimal fractionation schedule for stereotactic ablative radiotherapy (SABR)

Material and Methods: Syngeneic Lewis lung carcinomas were grown either orthotopically or subcutaneously in C57BL/6 mice and were irradiated with a single dose of 15 Gy to mimic SABR used in the clinic. Serial [18F]-misonidazole (F-MISO) positron emission tomography (PET) imaging, pimonidazole FACS analyses, hypoxia-responsive element (HRE)-driven bioluminescence, and Hoechst 33342 perfusion were performed before irradiation (d-1), at 6 hours (d0), 2 (d2), and 6 days (d6) after irradiation for subcutaneous and orthotopic lung tumors. For F-MISO, scan was performed 2 hr after the intravenous injection of F-MISO probe and the tumor-to-brain ratio (TBR) was analyzed.

Results: We observed that hypoxic signals were too low to quantitate for orthotopic tumors by F-MISO PET or HRE-driven bioluminescence imaging. In subcutaneous tumors TBR values were 2.87 ± 0.483 at d-1, 1.67 ± 0.116 at d0, 2.92 ± 0.334 at d2, and 2.13 ± 0.385 at d6, indicating that tumor hypoxia was decreased immediately after irradiation and returned to the pretreatment levels at d2, followed by a slight decrease by d6 post-radiation. Pimonidazole analysis also revealed similar patterns. By using Hoechst 33342 vascular perfusion dye and CD31 co-immunostaining, we found that there was a rapid and transient vascular collapse, which may have resulted in poor intratumoral perfusion of F-MISO PET tracer or pimonidazole delivered at d0 leading to decreased hypoxic signals at d0 by PET or pimonidazole analyses.

Figure 1

Fig. 1. Temporal changes in tumor hypoxia for subcutaneous tumors by F-MISO PET imaging. (A) Representative PET images demonstrating F-MISO uptake in subcutaneous tumor. Arrows indicate the tumor position. (B) A graph showing TBR values for an individual animal. (C) A graph showing the mean ± s.e.m. of TBR values (n = 5). 

Conclusion: We found tumor hypoxia levels to be returned to the pretreatment levels by 2 days after irradiation, hence supporting the current fractionation intervals of SABR being given at least 2 days. Our results also indicate that SABR may produce a rapid but reversible vascular collapse in tumors.

PV-0428
Factor 2.5 radiosensitivity difference determined by ex vivo γH2AX assay in prostate cancer patients

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Purpose or Objective: In previous study we showed that γH2AX assay in ex vivo irradiated tumour samples collected from cancer patients of various types correlates with known differences in radioresponsiveness. In the present study we aimed to apply the assay in a panel of prostate tumour
specimens to investigate whether it could allow discrimination of sensitive and resistant tumours of the same type. In addition we aimed to further explore the robustness of the method via investigating the potential impact of the tumour sampling on the reproducibility of the results.

**Material and Methods:** Tumour biopsies from prostate cancer patients undergone radical prostatectomy were cultivated in media for 24 h before irradiation (IR) with single doses and fixed 24 h post IR. The microenvironmental parameters were determined by addition of BrDU (perfusion) and Pimonidazole (hypoxia) to media prior to IR. Histological sections of previously paraffin-embedded material were stained for γH2AX and the foci were evaluated in viable, well oxygenated tumour areas. To investigate the heterogeneity of radiation response among the different patients, biopsies were irradiated with graded single doses (0, 2, 4, 6, 8 Gy) to determine the intratumoural sampling variability, biopsies from different tumour locations were irradiated with single dose of 4 Gy.

**Results:** In all the 15 patients currently analyzed we observed a linear dose-response of residual γH2AX foci. The slope of the dose-response expressed high heterogeneity among the different patients (slope values range: 0.83-2.27). Using the slope of the foci dose-response as a parameter of tumour radiosensitivity we could determine 3 patients subgroups, namely resistant, with slope values lower than the 25th percentile of the slope values distribution (<1.1); moderate, with slope values between the 25 and 75th percentile and sensitive, with slope values above the 75th percentile (>1.8). These results are consistent with previously observed slope values for very sensitive (e.g. seminoma, slope value >2) and resistant (e.g. GBM, slope value ~1) tumour types. ANOVA analysis of the residual foci values post 4 Gy IR evaluated in tumour cells form different parts of the same tumour revealed no significant differences in the foci value distributions.

**Conclusion:** We herein show for the first time that the γH2AX ex vivo assay is clinically feasible and able to detect differences in cellular radiation sensitivity among patients with the same tumour type. Our results suggest that intratumoural heterogeneity (potential source of sampling error) do not significantly affect the results of the assay. Taken together, this assay has a promising potential for individualized radiation oncology and prospective validation in different tumour types in relation to known tumour characteristics and patient’s outcome is warranted.

**PV-0429**

A 3D in vitro cancer model and imaging platform to measure proton radiation-induced cellular damage

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**Purpose or Objective:** The aim of the project is to present an in vitro 3D cellular platform capable of measuring radiation-induced cell damage at the cellular scale, enabling high-resolution image capture of cell response along the proton depth dose.

**Material and Methods:** A 3D cancer model of dimensions 17 mm x 17 mm x 5 mm (L x W x H) was developed for proton irradiation. The model comprises 1 million uniform distributed HT29 colon cancer cells within a type 1 collagen scaffold. The model was irradiated with 62 MeV proton spread out Bragg peak (SOBP) of 10 mm width. Samples were fixed after irradiation, set within agarose gel, processed via vibratome to 400 nm thickness slices, stained with markers for apoptosis (Caspase-3), DNA double strand breaks (53BP1) and hypoxia (CA9).

**Results:** Alamar blue assay proves the cell metabolism can maintain 1-5 days depending on seeding density. The cancer cells invade into stroma, form spheroid and show paracrine activity (vascular endothelial growth factor and epidermal growth factor receptor expression) and hypoxia gradients in 3D model. The measurement of DNA double strand breaks is achievable in 2D fluorescent microscopy but less easily resolvable in 3D imaging. The level of cell apoptosis along SOBP can be imaged and correlated to the actual position and dose. Figure below shows 1 million HT29 3D models are irradiated by 5Gy dose and fixed 24 hour after irradiation. The image position located at the proximal edge of the SOBP.

**Conclusion:** In this novel methodology of sample processing and well-controlled coordination system, correlation between the cell response of the 3D cancer model and proton dose distribution was possible. The fluorescent images show a clear difference in cell apoptosis signal response with depth dose, and in the 3D samples we could image a hypoxia gradient. Further work is underway to model LET within the 3D cancer model to be linked to cell response parameters, and to repeat the experiment under x-ray irradiation.

**PV-0430**

Late radiation enteropathy: do tissue cytokines play a protective role? A first-in-man study

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**Purpose or Objective:** Late radiation enteropathy affects 20% of prostate cancer survivors. Inflammatory processes may relate to its occurrence. We aimed to assess differences in the levels of intestinal mucosa cytokines between patients with side-effects after pelvic radiotherapy and healthy controls.

**Material and Methods:** Patients with GI symptoms developing after prostate radiotherapy and undergoing colonoscopy were recruited for this study. Controls were patients undergoing colonoscopy for polyp surveillance. All participants were free of bowel cancer. Colonoscopy was performed after standard preparation of the bowel with citramag and senna or Fleet prep. Biopsies were obtained for cytokine characterization and pathologic assessment as follows (Fig.1):

- (1) Two endoscopic directed biopsies were taken from an area where mucosal radiation lesion was present; if no mucosal change was obvious, biopsies were taken from the anterior rectal wall.
- (2) A second pair of biopsies was taken from normal looking mucosa as close as possible to the previous sampling site.