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) while 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 h 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.
Lymphocytes after neoadjuvant radiochemotherapy

Conclusion: Inflammatory response will be the object of further analyses.

Microbiota. Pathway analysis and modeling of the mucosal barrier or in keeping a normal balance of gut function of cytokines, either in the maintenance of the integrity of the epithelial barrier or in the regulation of the mucosal immune system may be important in the development of late radiation enteropathy. This may reflect a protective role of the microbiota in maintaining mucosal integrity.

Results: Recruitment ran from April 2014 to January 2015. 9 symptomatic patients, treated with prostate irradiation at least 2 years before and 6 healthy controls were recruited. Cytokine concentrations were higher in controls and in biopsies taken from normal tissue in the patients. Although patient samples from areas without disease had globally higher cytokine levels when compared to areas with disease, this was not significant. There was a trend to slightly higher histology scores in biopsies from irradiated tissues (table 1).

Table 1: Differences in global cytokine level analysis by panel and histology score

<table>
<thead>
<tr>
<th>Panel</th>
<th>Histology score</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemokine panel (20 cytokines)</td>
<td>None</td>
<td>0.001</td>
</tr>
<tr>
<td>Pro-inflammatory panel (30 cytokines)</td>
<td>None</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Conclusion: Cytokine levels are decreased in human tissues with late radiation enteropathy. This may reflect a protective function of cytokines, either in the maintenance of the mucosal barrier or in keeping a normal balance of gut microbiota. Pathway analysis and modeling of the inflammatory response will be the object of further analyses.

PV-0431
Changes of the density CD8+ tumour infiltrating lymphocytes after neoadjuvant radiochemotherapy

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Purpose or Objective: The aim of this retrospective study is to evaluate the effect of neoadjuvant radiochemotherapy on the density of CD8+ tumor infiltrating lymphocytes (TILs) of rectal adenocarcinoma, by comparison of the density of CD8+ TILs in endoscopic biopsies before and resection specimens after the therapy.

Material and Methods: In total 53 patients with locally advanced rectal cancer were studied retrospectively. Neoadjuvant treatment comprised 50.4 Gy/28 fractions external radiation with continual 5-fluorouracil. Four to six weeks after the radiochemotherapy, surgical resection was performed. Immunohistochemistry was applied to assess CD8+ expression in both the pretreatment biopsies and resected specimens.

Results: During radiochemotherapy 30 patients (57%) had increased the density of CD8+ TILs, in 18 patients (34%) decreased, in 1 patient there was no change, in 4 patients it was not possible to assess the dynamics of the density of CD8+ TILs (in 2 patients due to insufficient amount of tissue for immunohistochemical analysis and in other 2 patients due to pathologic complete response after radiochemotherapy). The median of follow-up was 75 months (6.3 years). In 2 patients resection with microscopic residual tumor (R1) was performed and for 51 patients radical resection with microscopically negative margins (R0) was performed. Downstaging after preoperative radiochemotherapy was observed in 34 patients (64%). Five-year overall survival was 56% (95%CI: 43-70%). The density of CD8+ TILs was not significant in Cox regression analysis (p=0.16) or log-rank test (p=0.16). According to chi-square test (p=0.37) there was no significant impact of the increase of the density of CD8+ TILs after radiochemotherapy on downstaging. The increase of the density of CD8+ TILs after radiochemotherapy was associated with a trend of 2.5 longer overall survival in comparison with patients with the decrease of the density of CD8+ TILs after radiochemotherapy.

Conclusion: In the present study we did not observe any predictive or prognostic significance of the density of CD8+ TILs in endoscopic biopsies before radiochemotherapy, in resection specimens after the radiochemotherapy or in changes of the density of CD8+ TILs after radiochemotherapy. The limitation of our study is the number of patients (53). It is not excluded that in a larger number of patients predictive or prognostic significance of the density of CD8+ TILs could be detected.

PV-0432
Mechanisms and abscopal effects of combined mRNA-based radioimmunotherapy in a syngenic mouse model.

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Purpose or Objective: Tumor metastasis and tumor immune evasion present major challenges of cancer treatment. Radiotherapy has been demonstrated to overcome the immunosuppressive tumor microenvironment and anecdotal reports suggest that local tumor irradiation alone may also exert systemic or abscopal anti-tumor effects by immune-response stimulation with subsequent control of non-irradiated tumor metastases. This study aimed to assess abscopal effects of radiation alone and in combination with an mRNA-based tumor vaccination in a syngenic mouse model.