

### Purpose or Objective

The highly proliferative hair follicles are sensitive to radiation and hair loss (alopecia) often develops. We found, local prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) pretreatment could reduce hair loss from radiation injury by preventing entry into catagen (regression phase) and telogen (resting phase) (Figure 1a). Whether and how anagen hair follicles attempt to repair themselves in response to ionizing radiation (IR) under PGE<sub>2</sub> local treatment has not been characterized. In this work, we try to investigate the proceeding regenerative process in hair follicle cells following radiation injury under prostaglandin treatment.

### Material and Methods

The dorsal hair of female C57BL/6 mice on postnatal day 32 when hair follicles were in the early full anagen (growth phase) was carefully shaved. Single dose of 8.5Gy was given from dorsal side 2 hours after dmPGE<sub>2</sub> (A stabilized derivative of prostaglandin E<sub>2</sub>, 16,16-dimethyl-PGE<sub>2</sub>) locally injected. Skin specimen was harvested at multiple time points after radiation exposure. H&E staining, Immunohistochemical analysis and double staining with hair follicle cell markers were used to monitor the dynamics of cell differentiation at different time points. The fluorescence ubiquitination cell cycle indicator (FUCCI) system, which can easily determine G1 and/or S/G2/M phases of the cell cycle through analyzes living cells in a spatio-temporal manner using a dual color scheme of orange and green, is used for evaluating cell cycle condition.

### Results

Severe dystrophic change of anagen hair follicles were observed after 8.5Gy radiation injury. We found anagen hair follicles were able to initiate spatially and temporally repair activities to restore their structure under PGE<sub>2</sub> pretreatment after radiation injury. The hair follicle bulge stem cells were not activated during the repair process. (Figure 1b) Continuous BrdU pulse labeling after PGE<sub>2</sub> local pretreatment revealed that the proliferation of hair follicle bulb matrix cells (hair follicle transit amplifying cells (TACs) ) was transiently halted (Figure 2a). Immunostaining of cell cycle marker of cyclin D showed decrease level after PGE<sub>2</sub> pretreatment. (Figure 2b). These suggested PGE<sub>2</sub> might reduce the radiosensitivity of TACs through cell cycle G1 phase arrest. We further took advantage of the fluorescence ubiquitination cell cycle indicator (FUCCI) system and confirmed the process.

Figure. 1

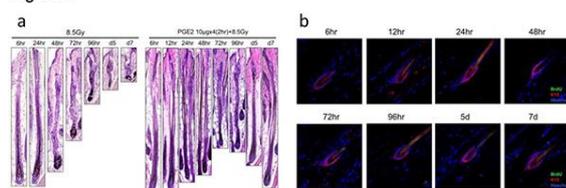
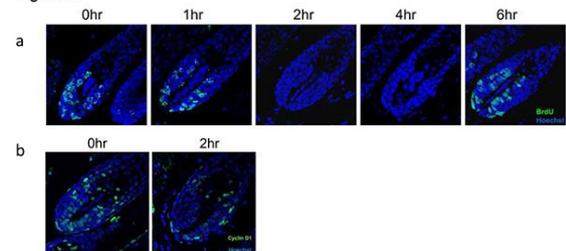


Figure. 2



### Conclusion

Under radiation injury, anagen hair follicles were able to initiate distinct repair activities to restore their structure after PGE<sub>2</sub> pretreatment through cell cycle modification.

Radiation-induced alopecia might be prevented by modulating cell cycle associated signaling to enhance spontaneous repair.

### PV-0539 Antidiabetic biguanides radiosensitize hypoxic cancer cells through a decrease in oxygen consumption

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### Purpose or Objective

The anti-diabetic biguanide drugs metformin and phenformin exhibit antitumor activity in various models. However, their radiomodulatory effect under hypoxic conditions, particularly for phenformin, is largely unknown. This study therefore examines whether metformin and phenformin as mitochondrial complex I blockades could overcome hypoxic radioresistance through inhibition of oxygen consumption.

### Material and Methods

A panel of colorectal cancer cells (HCT116, DLD-1, HT29, SW480, and CT26) was exposed to metformin or phenformin for 16 h at indicated concentrations. Afterward, cell viability was measured by MTT and colony formation assays. Apoptosis and reactive oxygen species (ROS) were detected by flow cytometry. Phosphorylation of AMP-activated protein kinase (AMPK) was examined by western blot. Mitochondria complexes activity and oxygen consumption rate (OCR) were measured by seahorse analyzer. The radiosensitivity of tumor cells was assessed by colony formation assay under aerobic and hypoxic conditions. The *in vitro* findings were further validated in colorectal CT26 tumor model.

### Results

Metformin and phenformin inhibited mitochondrial complex I activity and subsequently reduced OCR in a dose-dependent manner starting at 3 mM and 30  $\mu$ M, respectively. As a result, the hypoxic radioresistance of tumor cells was counteracted by metformin and phenformin with an enhancement ratio about 2 at 9 mM and 100  $\mu$ M, respectively. Regarding intrinsic radioresistance, both of them did not exhibit any effect although there was an increase of phosphorylation of AMPK and ROS production. In tumor-bearing mice, metformin or phenformin alone did not show any anti-tumor effect. While in combination with radiation, both of them substantially delayed tumor growth and enhanced radioresponse, respectively, by 1.3 and 1.5-fold.

### Conclusion

Our results demonstrate that metformin and phenformin overcome hypoxic radioresistance through inhibition of mitochondrial respiration, and provide a rationale to explore metformin and phenformin as hypoxic radiosensitizers.

### PV-0540 Tumor modifications recorded with IVIM and DCE-MRI after Neoadjuvant radiotherapy

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### Purpose or Objective

Neoadjuvant radiotherapy (NeoRT) improves tumor local control and facilitates tumor resection in many cancers. We hypothesized anti-cancer treatments (i.e. radiotherapy) modify tumor microenvironment and could potentially impact distant metastases occurrence.

Previously, we developed a pre-clinical model demonstrating an impact of NeoRT schedule and the timing of surgery on metastatic spreading (Leroi et al. *Oncotarget* 2015). Here, we aim to identify by fMRI non-invasive markers reflecting NeoRT related tumor microenvironment modifications that could predict the best timing for performing surgery and avoiding tumor spreading.

#### Material and Methods

To briefly delineate the NeoRT model, MDA-MB 231 tumor cells implanted in the flank of SCID mice were locally irradiated with 2x5Gy when tumor reached 100mm<sup>3</sup> and then surgically removed at different time points. We performed fMRI, Diffusion Weighted (DW) and Dynamic Contrast enhancement (DCE) - MRI, before RT and every 2 days between RT and surgery. 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. We performed IVIM (IntraVoxel Incoherent Motion) analysis to obtain information on intravascular diffusion, related to perfusion ( $F$ : perfusion factor) and subsequently tumor vessels perfusion. For DCE-MRI, we performed a T1 mapping with multiple TR and DCE acquisition with 200 repetitions of 3 sec each and gadolinium IV injection after 10 repetitions. We performed semi-quantitative analysis. We validated tumor perfusion by immunohistochemistry with injection of FITC-dextran IV 3 min before surgery and CD31 labelling. Human Ki67 was used for lung metastases labelling and quantification.

#### Results

After the tumor irradiation, we observed a significant and transient increase at day 6 (60% of the basal value ( $n=6$ ,  $p<0,05$ )) of  $F$  and  $D^*$  parameters related to perfusion. The other parameters of the DW-MRI, ADC and  $D$  presented no modifications. The sham irradiated tumors used as control showed no modifications of all fMRI parameters. At the same timing, 6 days post-radiotherapy, DCE-MRI significantly demonstrated a WashinSlope ( $n=13$ ,  $p<0,05$ ) increase. Immunohistochemistry confirmed the increase of tumor perfusion when surgery is performed at day 6. The sham irradiated tumors never demonstrated such changes. Finally, when surgery is performed on tumor increased perfusion measured by fMRI, it demonstrated a burst of lung metastasis compared to the other timings.

#### Conclusion

We showed a significant difference in perfusion-related parameters with fMRI and immunohistochemistry at a specific time point after NeoRT. These modifications are correlated with an increase of metastasis spreading related to surgery procedure. These results open new perspectives in the personalized medicine and MRI guided surgery timing after NeoRT.

#### PV-0541 Immune modulation by brachytherapy in peripheral blood

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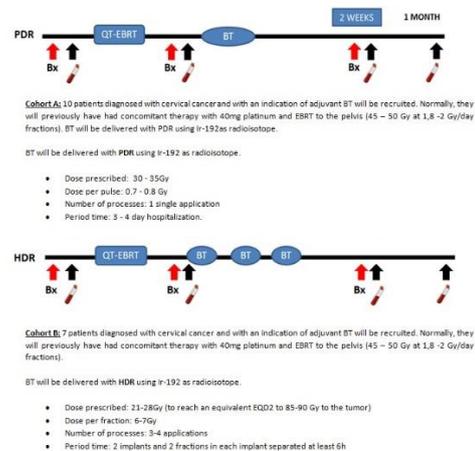
#### Purpose or Objective

Treatment with brachytherapy, either High Dose Rate (HDR) or Pulsed Dose Rate (LDR), is an effective treatment with consolidated results in cervical cancer, but its effect on the immune system is unknown. The effect of HDR-BQT as well as SBRT or IORT, can generate an individual immunity that can lead to lasting systemic responses, causing superior tumor control in these patients. These changes should be greater in HDR than in LDR. Our objective was to compare the effect on the immune system of both treatment in cervical cancer with the idea

of being able to find predictive and prognostic factors for diagnosis in these patients.

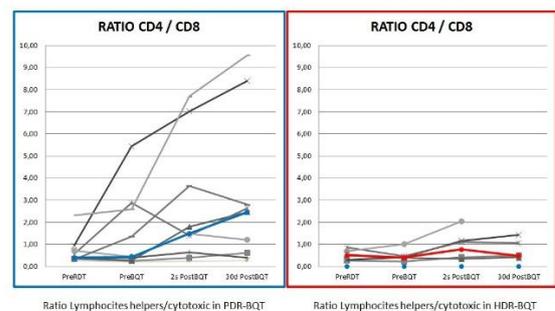
#### Material and Methods

We studied immunological factors in peripheral blood of 17 patients diagnosed with cervical cancer, 10 were treated with LDR-BQT and 7 with HDR-BQT. Four blood samples were obtained at different times of chemotherapy + radiotherapy and LDR / HDR-BQT. A blood sample was obtained prior to the start of treatment with RT, second sample before the start of the BQT, another sample at 2 weeks after the end of the BQT and a fourth sample one month after the end of the treatment. These samples were studied by flow cytometry in 3 panels. In the first panel the lymphocyte phenotype is studied; B lymphocytes, T lymphocytes, NK cells and monocytes. In another panel the regulatory T cells (Treg) were studied and in the third panel the suppressor cells derived from myeloid cells (MDSC). Subsequently, the effect on the tumor microenvironment will be analyzed with biopsies taken at different times of treatment.



#### Results

In the phenotyping panel there was an increase in TCD8 cell values and a decrease in TCD4 cells in BQT-HDR with a CD4 / CD8 ratio favorable to the BQT-HDR arm. An increase of NK cells in HDR-BQT was observed. At the Treg cell level there was a decrease in the HDR-BQT arm. In the MDSC panel an increase of the CD4 + CD45Ra-CD25 + and FOXP3 + cells in the HDR-BQT arm was evidenced.



#### Conclusion

Radiation doses delivered with HDR-BT in cervix cancer patients could trigger immune stimulation by modulating immune cells in plasma and tumor microenvironment. Deciphering immune-responses to treatment in cervix cancer patients could introduce new biomarkers helpful for treatment choice in the future.