Conclusion: Herein c-Myc acts as a key master regulator of in vitro migration, invasion and radioresistance. In fact, c-Myc depletion alone seems to be sufficient to block the in vitro pro-metastatic abilities and to radiosensitize ERMS cells. In addition, our data suggest c-Myc as important, but not essential, in controlling the molecular machinery responsible for cancer neo-angiogenesis. In conclusion these data strongly suggest that the targeting of c-Myc can be tested as a promising strategy for an anti-cancer therapy.

EP-2063
Apopotic pathway activation in prostate neoplastic cells after 12 Gy-IORT
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Purpose or Objective: To evaluate apoptotic pathways involved in prostate cancer treated with intraoperative radiotherapy (IORT) with 12 Gy, studying the effects on cancer cells, prostatic intraepithelial neoplasia (PIN) and normal cells

Material and Methods: Since 2005, 111 patients treated at University Hospital of Novara, Italy with local advanced prostate adenocarcinoma were treated with radical prostatectomy and 12 Gy IORT followed by 50 Gy postoperative radiotherapy. In this setting, we selected a sample of 10 patients for a preliminary feasibility study. Selection criteria for this phase were: no neoadjuvant hormone therapy, Gleason score > 7. Proteins involved in the apoptotic cascade (Bax, Caspases-3 and -9) were studied before and after 12 Gy single shoot in neoplastic cells, high grade PIN areas and in normal prostate cells. Immunofluorescent detection of antigens (anti-Bax, anti-caspases-3 and -9), were performed on biopct sample and on surgical specimens 5-mm slices. On surgical specimens there were also detected Bcl-2, and Ki-67 with immunohistochemical analysis. A count of positive spots for immunofluorescence (Bax+, Caspases-3 and -9+/all nuclei, 40x magnification) was performed on tumor cells, PIN, healthy tissue areas. Bax and caspases immunofluorescent positivity was compared in different areas and in neoplastic areas before and after single shoot high dose

Results: A significant increase in Bax, Caspases-3 and -9 expression was detected in tumor and PIN areas comparing IORT treated and untreated samples (p<0.05). After 12 Gy single dose, healthy areas expressed significantly lower level of Bax and caspases positive with respect to neoplastic cells (p=0.0001), while in PIN areas, Bax positive cells were significantly more present than in neoplastic areas (p=0.0001). Mean Bcl-2 in neoplastic cells is 17% (range: 1-23), mean Ki-67 in neoplastic area is 4.5% (range: 1-17). With multivariate analysis, we find that cancer cells with Ki-67 ≥ 8% show a trend toward greater expression of Bax (p=0.0641)

Conclusion: After 12 Gy irradiation, Bax and caspases resulted overexpressed in tumor and PIN cells, in particular in prostate cancer with higher proliferation index. PIN areas seem to be more radiosensitive than neoplastic areas and healthy cells do not activate apoptosis after single shoot, showing an intrinsic radioresistance. This preliminary study represents the basis for an extensive work in which we would correlated clinical parameters with pathology and apoptotic factors. In fact, the comprehension of these relationships could allow to better understand the mechanisms of high dose per fraction and, radioresistance in order to personalize treatments

EP-2064
Radiation induces metabolic switch to lactate production to support tumour cell survival
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Purpose or Objective: Purpose: Radiation treatment of tumor cells resulted in a reduction of endogenous ATP levels. Aim of this study was to elucidate the molecular scenario standing behind this observation.

Material and Methods: Endogenous ATP-levels were determined by ATP-ELISA. HIFα, PDK1, LDH and PDH expressions were visualized by western blotting. Lactate production was quantified by lactate-assay. Cellular survival was proved by clonogenic survival assay.

Results: Results: Ionizing radiation induced expression of Hif1 alpha even at clinical relevant doses of 2 Gy. Hif1alpha induced activation of mitochondrial PDK1, which results in PDK1 dependent phosphorylation of pyruvate dehydrogenase (PDH). PDH is responsible for conversion of pyruvate to acetyl-CoA, which fuels the TCA cycle. Thus, irradiation blocks TCA cycle and mitochondrial activity. Simultaneously Hif1alpha induced expression and activity of lactate dehydrogenase (LDHA) to convert glucose to lactate. Indeed we observed a clear increase in lactate production in tumor cell lines in response to irradiation. Furthermore, inhibition of PDH activity was associated with mitophagy and ATP-depletion, which explains the radiation induced ATP drop down. In addition, this radiogenic switch to lactate production reduced production of mitochondrial derived radicals and increased cellular radio-resistance. Pretreatment with the Hif1 alpha inhibitor BAY87-2243 prevented the radiogenic switch to lactate metabolism and radio-sensitized the tumor cells. In addition, tumor cells are strictly dependent from high glucose supply after irradiation and can be radio-sensitized by blockage of radiogenic glucose uptake with glucose transporter SGLT inhibitor Phlorizin.

Conclusion: In summary, we could show, that tumor cells switch in a Hif1 alpha dependent manner to anaerobe glucose metabolism to generate ATP, which renders cells radio-resistant. Blockage of Hif1 alpha stabilization or blockage of glucose uptake radio-sensitized tumor cells.

EP-2065
Effects of spontaneous γH2AX level on radiation-induced response in human somatic cells
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Purpose or Objective: Phosphorylated histone H2AX (γH2AX) foci are well-known markers of DNA double-strand breaks in human cells. Spontaneous γH2AX foci form on unrepaired DNA double strand breaks, shortened telomeres and sites with altered chromatin conformation. The presence of such permanent γH2AX foci in cell is an important component of epigenetic background and potentially lead to the activation of DNA repair system. The objective of this study was to analyze the effects of spontaneous γH2AX level on radiation-induced response in human somatic cells.

Material and Methods: Spontaneous γH2AX foci and radiation-induced micronuclei were analyzed in peripheral blood lymphocytes of 54 healthy individuals after exposure to 2 Gy ionizing radiation in vitro. Further, a transcriptome analysis was performed using gene expression microarrays in lymphocytes of two sub-groups of individuals: 1)
radiosensitive individuals with low spontaneous level of γH2AX foci (n=3) and 2) radioresistant individuals with high spontaneous level of γH2AX foci (n=3).

**Results:** An inverse correlation was found between the spontaneous level of γH2AX foci and the frequency of micronuclei after irradiation (R=0.37, p=0.025). After gene expression analysis with microarrays, several genes were identified whose differential expression could be associated with an efficiency of DNA repair and radiation sensitivity. XRR1 gene with unknown functions, recently associated with radiosensitivity in tumor lines, was down-regulated both before and after irradiation in radioresistant group. Furthermore, in unirradiated samples of radiosensitive individuals thrombospondin gene (THBS1), well-known radiosensitizer, was down-regulated. However, several genes were significantly up-regulated, including HERC2, important player in the assembly of DNA repair foci, and histone genes (H1, H2A, H4). After irradiation, several DNA repair genes (WHSC1, POLN, ERCC5, DCLRE1C) were significantly up-regulated, but EIF2A and PNPLA5 genes, involved in apoptosis and autophagy, were down-regulated in radiosensitive group. This is consistent with low levels of apoptosis and increased proliferation in lymphocytes of these individuals.

**Conclusion:** The obtained results indicate that spontaneous γH2AX foci activate DNA damage response in human somatic cells and provide opportunities to clarify the role of the expression of identified genes in the formation of chromosomal aberrations in human cells after exposure to radiation.

**EP-2066**

Phospholipase Cε as a biomarker of prostate cancer radioresistance

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Purpose or Objective: Radiotherapy is a curative treatment option in prostate cancer. Nevertheless, many men with prostate cancer develop recurrence of their disease. Identification of the predictive biomarkers and signaling mechanisms indicative of tumor cell radioresistance bears promise to improve cancer treatment. In our study we show that Phospholipase C epsilon (PLCε) might contribute to prostate cancer radioresistance.

**Material and Methods:** Gene expression profiling of prostate cancer cells and their radioresistant derivatives, within Western blot analysis to assess PLCε expression in the parental and radioresistant cell lines and in cell cultures after irradiation, radiobiological cell survival analysis of the cells with genetic modulation of PLCε expression by siRNA or CDNA transfection as well as chemical inhibition of PLCε activity, fluorescent microscopy to analyze co-expression of PLCε with other markers of radioresistance. Normal 0 21 false false false EN-US X-NONE X-NONE

**Results:** The results of gene expression analysis, which were validated by western blotting revealed significant upregulation of PLCε in prostate cancer radioresistant cells that can also be seen after irradiation of the parental cells with a single dose of 4 Gy. Radiobiological survival assays demonstrated that siRNA-induced knockdown of PLCε activity by Edofosine leads to prostate cancer cell radioreosensitivity. In contrast, overexpression of PLCε in cells transfected with plasmid DNA results to an increase in cell radioresistance. Microscopic analysis revealed a high expression level of β-catenin in prostate cancer cells overexpressing PLCε.

**Conclusion:** These results indicate that PLCε plays a role in prostate cancer radioresistance that can be mediated through activation of the WNT/β-catenin signaling pathway.

**EP-2067**

The adhesion of tumor cells to endothelial cells is increased by photon irradiation

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Purpose or Objective: In general the prognosis for cancer patients is poor even though only 10% die from the primary tumor. The majority of the deceases are due to metastasis. Given the fact, that more than 70% of cancer patients receive radiotherapy it seems important to clarify if radiation is involved in initial steps of the metastatic cascade - despite of innumerable clinical studies that confirm no enhanced risk of metastasis after radiotherapy. In this project we investigated whether the irradiation with photons increases the adhesion of cultured tumor cells (TC) to a layer of endothelial cells (EC) macroscopically and whether this might be caused by the induction of adhesion proteins.

**Material and Methods:** The experiments were performed with glioblastoma (U87, U373) and breast cancer cell lines (MDA-MB-231, MCF7), and with primary HUVEC cells. The cells were irradiated with 0, 0.5, 2, 4, or 8 Gy. Adhesion of TC to EC, both irradiated or not, was determined with 2 different methods: the VybrantTM cell adhesion assay and the ibidi pump system that allows to mimic the physiological blood stream in the vasculature. In addition, the expression of the adhesion-related proteins E-selectin, VCAM1, ICAM1, N-cadherin, integrin β1, and PECAM1, 4h after irradiation with 4 Gy, was analyzed by qRT-PCR and by Western blotting.

**Results:** Irradiation increased significantly the adhesion of TC to EC. With glioblastoma cells the highest increase of about 40% was observed when both cell types were irradiated. In contrast, with breast cancer cells the highest effect of about 25% was obtained for irradiated TC in combination with non-irradiated EC. Analysis of the expression patterns in all cell types revealed an significant increase of adhesion proteins after irradiation in more than 80% of the experimental data sets.

**Conclusion:** We assume that the irradiation of tumor cells as well as of endothelial cells with photons might enhance adhesive interactions of these cells and thereby might promote the first steps of metastasis. Since clinical studies reveal no enhanced risk of metastasis due to irradiation we speculate that the therapeutic effect of radiotherapy might be additionally enhanced when the induced stickiness could be blocked effectively.

**EP-2068**

Effect of a 0.2 T magnetic field during radiation on DNA damage and repair in prostate cancer cells


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