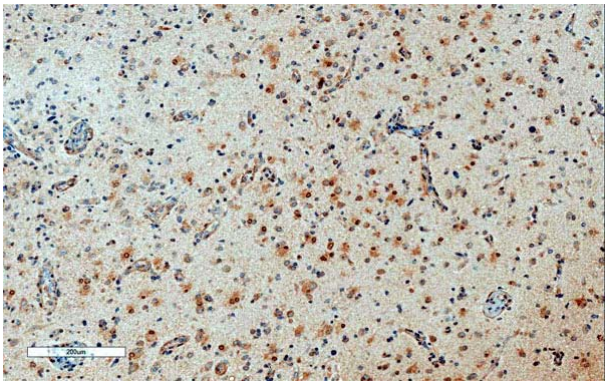
Purpose or Objective: The standard treatment regimen of patients with primary glioblastoma multiforme (PGBM) consists of neurosurgery, radio- and chemotherapy. Despite this multimodal treatment the overall survival of patients with PGBM is still approximately 15 months.
The stress-inducible heat shock protein 70 (Hsp70) contributes to tumor cell survival and is associated with poor prognosis, metastasis and therapy resistance. Therefore, the aim of this study is to analyze Hsp70 in PGBM tumor samples as a future prognostic biomarker and possible therapy target.

Material and Methods: Formalin fixed paraffin embedded (FFPE) sections of 44 human PGBM patients (isocitrate dehydrogenase wildtype) were analyzed by immunohistochemistry for Hsp70 (cmHsp70.1, IgG1, multimmune GmbH, Munich, Germany). Taking the intensity of Hsp70 staining into account, quantitative expression analysis of tumor cells with stained cytoplasm was performed. Two categories of Hsp70 staining were defined: Up to 40% and more than 40% positive tumor cells within the tumor regions. The Hsp70 immunoreactivity was correlated with the survival of the patients using the Cox regression analysis.

Results: Preliminary data show that the median survival of PGBM patients can be predicted by the Hsp70 immunoreactivity of the tumor cells. Regression analysis showed that patients with Hsp70 expression of more than 40% have a higher risk of disease progression with a hazard ratio of 2.59 (p= 0.045).



Hsp70 expression in FFPE IHC section (Hsp70 positive tumor cells are brown)

Conclusion: These data provide the first evidence that Hsp70 expression in FFPE sections of PGBM patients is associated with disease progression. Moreover, measuring Hsp70 in FFPE sections of PGBM patients before radiotherapy treatment may be used as biomarker for the success of the therapy. The independency of Hsp70 expression and O6-methylguanin-DNA methyltransferase (MGMT) is currently under investigation.

EP-2052

Expression of molecular biomarkers in wound drainage fluids: a pilot study in head and neck cancer

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Purpose or Objective: In recent years, it has been suggested that wound drainage fluids (WDF) of patients operated for head and neck squamous cell carcinoma (HNSCC) may be characterized by molecular biomarkers with potential prognostic and predictive value. The detection of adverse features in the early perioperative setting could possibly lead to a refinement of current adjuvant treatments in high-risk patients. The purpose of our study is to report on the feasibility and preliminary results of a pilot prospective study on WDF analysis in HNSCC.

Material and Methods: 14 consecutive surgically resected HNSCCs were studied. WDF were collected 1 day and 3 days after surgery from the cancer operative bed (COB). In 5 patients, WDF was collected also from free flap donor site (FFDS). WDF were centrifuged for 15 min at 3500 rpm, then divided in aliquots and stored at -80°C until analysis. The aim of the present study was to evaluate the expression of factors involved in tumor growth and progression 1 day and 3 days after surgery. EGF, VEGF, SDF-1 and osteopontin levels were measured in WDF using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Each sample was analyzed in duplicates and then averaged for a mean value. Quality control pools of low, normal, or high concentrations for all parameters were included in each assay. The obtained results were expressed as pg/ml (EGF, VEGF, SDF-1) or ng/ml (osteopontin).

Results: A mean of 67 ml of WDF from COB and 51 ml from FFDS at day 1, and 42 ml from COB and 20 ml from FFDS at day 3 were collected for each patient. EGF expression was significantly reduced from day 1 to day 3 after surgery both in COB (140.7±10.55 vs. 45.12±13.35 pg/ml, $p<0.001$) and in FFDS (157.1±4.08 vs. 95.59±32.89 pg/ml, $p<0.05$). VEGF expression increased from 1 to 3 day both in COB (1277.74±64.54 vs. 1616.81±151.4 pg/ml, $p<0.05$) and in FFDS (1227.51±19.39 vs. 1400.25±77.66 pg/ml, $p<0.05$). The expression of markers of invasiveness and metastasis increased from day 1 to day 3: osteopontin expression significantly increased from day 1 to day 3 both in COB (9.97±0.68 vs. 16.87±0.56 ng/ml, $p<0.001$) and in FFDS (9.51±1.23 vs. 15.83±1.08 ng/ml, $p<0.01$). SDF-1 expression increased from day 1 to day 3 in COB (646.8±65.39 vs. 1084.22±148.8 pg/ml, $p<0.05$). No differences in SDF-1 expression were detected in FFDS.

Conclusion: Preliminary data from pilot study evidenced that microenvironment induced by surgery favors residual tumor cell proliferation and progression. Growth factor expression is higher early after surgery (24 hours); on the contrary, expression of markers of invasiveness and metastasis increases from day 1 to day 3 after surgery. The few samples of WDF from FFDS do not allow to evidence differences of biomarkers expression between COB and FFDS.

EP-2053

In-vivo imaging of rat leukocytes redistribution after pelvic irradiation

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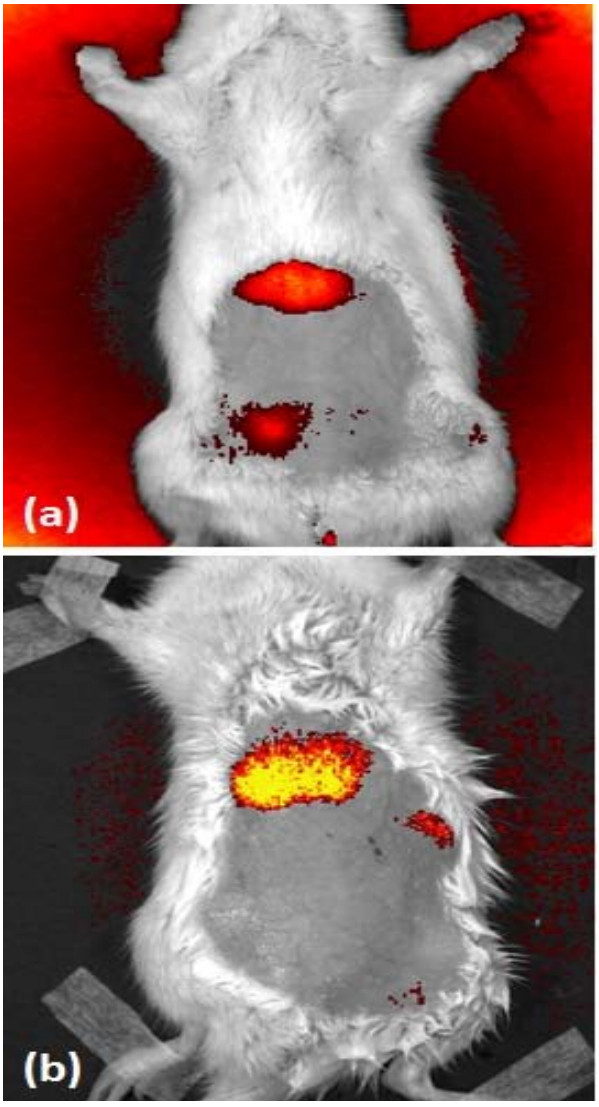
Purpose or Objective: Hematologic toxicity and in particular decrease in the peripheral blood leukocyte and lymphocyte count is an important side effect of pelvic radiation therapy. The aim of this study was to investigate the kinetic of the redistribution of circulating leukocytes after pelvic irradiation in a animal model with in vivo non-invasive imaging modality.

Material and Methods: A rat model was used to investigate a possible selective accumulation of circulating lymphocites to specific anatomical districts after radiation treatment focused to the urinary bladder. Eight Fisher rats were adoptively transferred with 4x107 VivoTag-750-labelled syngeneic primary splenocytes at two hours before the bladder irradiation. Two of eight rats were used as controls. Animals were transurethrally catheterized to allow contrast agent instillation. A kV cone beam computed tomography (CBCT) was acquired for each rat, to precisely deliver 6 MeV monofraction photon field. Rats were divided into three groups (n=2/group) receiving different levels of dose: 15, 20 and 25 Gy. A bolus thickness equal to 1cm was positioned on the rat skin surface in the pelvic region. Ultrasound images of the pelvic region were acquired at baseline, at 2, 4 and 6 days after irradiation to monitor thickness variations of the bladder wall tissue. In vivo fluorescent imaging was used to evaluated accumulation sites of labelled leukocytes.

Results: A significant increase in the bladder wall thickness was found 4 days after irradiation in animals treated with a dose equal to 25 Gy. A fluorescent signal, secondary to labelled splenocytes accumulation, emerged in the liver and lymph nodes of all adoptively transferred rats, 2 and 6 days after irradiation, as expected. A modest specific signal (30% increase) at the bladder level resulted only in two animals receiving the higher dose (Figure 1.a), when compared to the non-irradiated (Figure 1.b). No specific fluorescent signal was detected at the bladder levels in animals treated with 20 and 15 Gy.



Conclusion: The relocalization of peripheral leukocytes in the damaged tissue depends on the radiation dosage and it may be evaluated by means of a non-invasive imaging technique. Further analyses are currently ongoing.

EP-2054

Expression of DNA-PK in squamous cell lung cancer has gender differences and depends on smoking

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Purpose or Objective: Lung cancer is one of the most frequent and deadly types of cancer in Europe. Several aspects of non-small cell lung cancer (nsclc) in men and women continue to indicate potential male-female differences. Among these, higher treatment responses to current therapies in women are supposed, since women have better prognosis in any stage of the disease. In most stages of nsclc cytotoxic anti-cancer therapy (radiotherapy, chemotherapy) is used. It is known that treatment efficacy of cytotoxic anti-cancer therapy depends on tumor DNA-repair. Therefore, the aim of this study was to evaluate gender differences in the expression of DNA repair enzyme DNA protein kinase (DNA-PK).

Material and Methods: Surgically excised nsclc tissues (n=111, 50 adenocarcinomas, 61 squamous cell carcinomas) were examined for DNA-PK expression. After immunohistochemistry, the staining intensity of DNA-PK was quantified using an arbitrary score ranging from 0 (no staining) to 3 (strong signal). Also, the proportion (%) of DNA-PK positive (DNA-PK+) tumor cells was determined. All parameters were examined by 2 independent researchers in 10 randomly chosen microscopic fields (magnification x40).

Results: Immunohistochemical parameters were examined by 2 independent researchers whose results were in good accordance ($p<0.0005$). Staining intensities of DNA-PK and the proportion of DNA-PK+ tumor cells varied, being in the whole nsclc group 2.4±0.4 (mean±SD) and 86.3±9.1% respectively. There were no significant gender differences in adenocarcinoma. However, we detected significant differences among nsclc patients with squamous cell carcinoma. Both, DNA-PK staining intensity and the proportion of DNA-PK+ tumor cells were significantly higher in men than in women, 2.5±0.3 and 86.3±8.8% vs 2.1±0.6 and 79.6±11.9% respectively (DNA-PK intensity: $p<0.01$; DNA-PK+ proportion: $p=0,03$). Additionally, we found that in squamous cell carcinoma, the expression of DNA-PK depends on smoking and pack-years. There was a correlation between pack-years and DNA-PK intensity ($p=0.04$), as well as between pack-years and the proportion of DNA-PK+ tumor cells ($p=0.04$).

Conclusion: Expression of DNA-PK in squamous cell lung cancer has gender differences and depends on smoking. Significantly lower expression of tumor DNA-PK was found in women with this histological subtype of nsclc. Latter might be one of the reasons why cytotoxic anti-cancer therapy is more efficacious in women than in men. In further studies, the combination of DNA repair inhibitors and cytotoxic anti-cancer therapy should be tested.

EP-2055

Fibro-inflammatory circulating proteins as biomarkers for response in locally advanced rectal cancer

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