timing of surgery and the RT schedule could influence tumor dissemination and subsequently patient overall survival. We demonstrated the impact of NeoRT on metastatic spreading in a Scid mice model. After an irradiation of 2x5Gy, we show more metastasis in the lung when the mice are operated at day 4 compared to day 11 (1). Here, our aim is to evaluate with functional MRI (fMRI) the impact of the radiation treatment on the tumor microenvironment and subsequently to identify non-invasive markers helping to determine the best timing to perform surgery for avoiding tumor spreading.

Material and Methods: We used two models of NeoRT in mice we have previously developed: MDA-MB 231 and 4T1 cells implanted in the flank of mice (1). When tumors reached the planned volume, they are irradiated with 2x5 Gy and then surgically removed at different time points after RT. Diffusion Weighted (DW) -MRI was performed every 2 days between RT and surgery. For each tumors 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, in the 3 main directions. We also performed IVIM (IntraVoxel Incoherent Motion) analysis, in the aim to obtain information on intravascular diffusion, related to perfusion (F: perfusion factor) and subsequently tumor vessels perfusion.

Results: With the MBA-MB 231 we observed a significant increase of F at day 6 after irradiation than a decrease and stabilization until surgery. No other modifications of the MRI signal, ADC, D or D* were observed. We observed similar results with 4T1 cells, F increased at day 3 than returned to initial signal (fig 1). The difference in the peak of F can be related to the difference in tumor growth between MBA-MB 231 in four weeks and 4T1 in one week.

Conclusion: For the first time, we demonstrate the feasibility of repetitive fMRI imaging in mice models after NeoRT. With these models, we show a significant difference between the pre-irradiated acquisition and day 6 or day 3 for perfusion F. This change occurs between the two previous time points of surgery demonstrating a difference in the metastatic spreading (1). These results are very promising for identifying noninvasive markers for guiding the best timing for surgery.


EP-2050
The assessment of fractal dimension with Dual Energy CT gives information on lung cancer biomarkers
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Purpose or Objective: To assess whether texture analysis of images obtained with Dual Energy CT (DECT) is related to KRAS and Ki-67 lung cancer biomarkers.

Material and Methods: A retrospective review (May 2013 - January 2015) of 125 lung cancer patients with lung GSI (Gemstone Spectral Imaging) and perfusion CT imaging on a DECT was fulfilled. For 25 of them, the fraction of Ki-67 positive-tumour cells was analysed and for 19 patients KRAS-positive (mutation detected) or KRAS-negative (mutation not detected) character was evaluated (11 positive, 8 negative). DECT examination was performed on a Discovery CT 750 HD scanner (GE Healthcare, USA).

For the perfusion exam, blood volume, blood flow and permeability-surface studies were analyzed. At GSI exam, images related to absorption in Hounsfield units (HU), iodine concentration and monochromatic virtual images were reconstructed at 40, 60, 80, 100, 120 and 140 keV were assessed. Tumour fractal dimension was measured with the use of Map fractalcount plug-in for ImageJ (National Institute of Health, USA) software.

After extraction of DNA from paraffin embedded tissue using QIAamp DNA Investigator Kit (Qiagen), analysis of the KRAS gene exons 2 (codons 12/13) and 3 (codon 61) were performed in order to identify possible associated mutations with real-time PCR kit cOBAS® KRAS Mutation Test (Roche Diagnostics, SL).

T-Student test or U Mann-Whitney test were used to compare differences between KRAS-positive from KRAS-negative cohorts. Pearson correlation coefficient was used to study linear relationship between fractal dimension and the fraction of Ki-67 positive-tumour cells.

Results: Best result (p=0.02) for distinguishing KRAS-positive cohort was obtained for lesion fractal dimension at 140 keV virtual image. This parameter showed an AUC=0.80. It was predictive of KRAS-positive with 90.9% sensitivity and 75.0% specificity for a fractal dimension threshold of 2.332. There was a correlation of lesion fractal dimension in blood volume image and the fraction of Ki-67 positive-tumour cells (p=0.04).

Conclusion: Ki-67 positive-tumour cells and KRAS-positive biomarkers lead to tumour heterogeneity that modify radiographic image. Fractal dimension parameter quantifies such imaging heterogeneity and could allow to differentiate them.

A higher fractal dimension (higher heterogeneity) of lesion at virtual monochromatic images is measured for KRAS-positive mutation, while a higher fraction of Ki-67 positive-tumour cells is associated with a more homogeneous blood volume at perfusion.
Patients. The purpose of our study is to report on the redistributed metastasis after surgery. The aim of this study was to investigate the kinetic of the hematologic toxicity and in particular decrease in the peripheral blood leukocyte and lymphocyte count is an important side effect of pelvic irradiation. 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.

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).

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-methylguanine-DNA methyltransferase (MGMT) is currently under investigation.

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 consecutively 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. EGFR, 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 (1227.74±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 irradiation 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.