Purpose or Objective: The standard treatment regimen of patients with primary glioblastoma multiforme (GBM) consists of neurosurgery, radio- and chemotherapy. Despite this multimodal treatment the overall survival of patients with GBM 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-methylguanine-DNA methyltransferase (MGMT) is currently under investigation.

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Expression of molecular biomarkers in wound drainage fluids: a pilot study in head and neck cancer
M. Sottini1, M. Mangoni1, P. Bonomo1, A. Deganello2, A. Javarone3, T. Guaitieri2, I. Desideri1, M. Loi1, I. Meattini2, F. Piai1, L. Liv1
1University of Florence, Experimental and Clinical Biomedical Sciences, Firenze, Italy
2University of Florence, Department of Surgery and Translational Medicine, Firenze, Italy

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 days both in COB (1277.74±64.54 vs. 1616.81±151.4 pg/ml, p=0.05) and in FFDS (1277.51±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 (5.1±1.23 vs. 15.83±1.08 ng/ml, p<0.01). SDF-1 expression increased from day 1 to 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.

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In-vivo imaging of rat leukocytes redistribution after pelvic irradiation
1San Raffaele Scientific Institute, URI/Urology, Milan, Italy
2San Raffaele Scientific Institute, Radiotherapy, Milan, Italy
3Fondazione Centro San Raffaele, Medical Physics, Milan, Italy
4San Raffaele Scientific Institute, Experimental Imaging Center- Medical Physics, Milan, Italy
5San Raffaele Scientific Institute, Experimental Imaging Center, Milan, Italy
6San Raffaele Scientific Institute, Experimental Imaging Center, Milan, Italy
7San Raffaele Scientific Institute, Lymphocyte Activation Unit- Immunology- Transplantation and Infectious Disease Division, Milan, Italy
8San Raffaele Scientific Institute, Unit of Cellular Immunology, Milan, Italy
9San Raffaele Scientific Institute, Medical Physics, Milan, Italy

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.