EP-0997 Polyphenols of Akt and EZH2 as predict factor of radiochemotherapy in patients with glioblastoma (GBM)  
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Purpose/Objective: GBM is most common primary brain tumor and represents an important challenge for clinicians. These neoplasms are resistant to radio-chemotherapy. This might be explained by the fact that the interaction between tumor and microenvironment is important for radio-resistance in tumor radioreistance through angiogenesis, hypoxia and immunosuppression, or an intrinsic radioreistance of cancer stem cells. A molecular analysis in tumor samples or peripheral blood of basal activation of different signaling pathways potentially involved in radioresistance could be of clinical interest. Phosphatidylinositol 3-kinase/protein kinase B (Akt) pathways serve to block apoptosis, keeping cells alive in very toxic environments such as chemotherapy and ionizing radiation. Polycycl group (PCG) proteins mediate gene silencing through histone post-translational modifications. PCG function is crucial for neural stem cell self-renewal. Recent evidence indicates that PCG genes are also required for cancer stem cell (CSC) propagation in neural tumors. In this study we evaluated the different genetic profile of Akt and EZH2 and clinical response to treatment in patient affected by GBM.

Materials and Methods: Our plan is to analyze fifty patients with GBM treated with Radio-chemotherapy (RT-CT) with temozolomide. Time to progression (TTP) after surgery or biopsy and overall survival (OS) will be used as clinical end-points to be correlated with polymorphisms of Akt and EZH2. DNA is extracted by proteinase K digestion. SNP genotyping was performed with the ABI PRISM 7900HT Sequence Detection System using primers and probes designed with Methyl Express software (v. 1.0). DNA samples (1-20 ng) from cancer tissue were amplified I multiplex Real-Time PCR. In addition, by quantifying them a 25- fluorescent signals of the VIC and FAM probes, which specifically annealed to the two allelic sequences, the SDS software (v. 2.1) determined the allelic content of each sample. For the Quantitative Methyl Specific PCR, the Bisulfite treatment of DNA was performed using the Methyl SEQr Bisulfite system according to manufacturer’s protocol. Following bisulfite treatment, the converted DNA was amplified using primers designed for the altered sequences.

Results: preliminary data is available for nineteen patients for polymorphisms of Akt after a follow-up of three - thirty-six months. The Akt1*3 wild-type genotype was detected in 12/19 of samples and the heterozygous genotype was found in 7/19. We also evaluated Akt1*4 polymorphisms, and the only 2 patients that showed mutant genotype had the longest survival time. The same analysis will be performed in 42 peripheral blood in pts underwent to the same genotype had the longest survival time. The same analysis will be performed in 42 peripheral blood in pts underwent to the same treatment for GBM, findings will be available for the ESTRO meeting in May.

Conclusions: This study is currently ongoing, we actively accruing new cases and we are waiting for the data of peripheral blood. This preliminary analysis appears to indicate allelic discrimination of SNPs is sensitive and reproducible method. The screening of cell line genotypes may add to the prognostic value of histopathology samples.

EP-0998 Conformal radio- and chemoradiotherapy in the management of high-grade glioma patients  
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Purpose/Objective: Evaluation of 5-year treatment outcomes in high-grade (grade III-IV) brain glioma patients administered conformal radio-(chemo)radiotherapy.

Materials and Methods: The study included 261 patients 22-74 years of age with Karnofsky performance scale of ≥ 50%. Fifty-four of them had pathological diagnoses of grade III anaplastic astrocytoma (AA), 17 grade III anaplastic oligoastrocytoma or grade III anaplastic oligodendroglioma (A/AOD), and 190 - grade IV glioblastoma (GBM). In 2005 - 2010, 114 of them received a course of postoperative conformal radiotherapy at a single target dose of 1.8-2.6 Gy up to total target doses (TDD) of 54-60 Gy, and 147 patients in 2007 - 2011 underwent a preoperative course of conformal chemoradiotherapy with temozolomide 75 mg/m² an hour before radiation treatment during the first and the last 2 weeks of radiotherapy at the same doses. The patient survival was evaluated using the data of Belarusian Cancer Registry as of November 15, 2012 and calculated with Kaplan-Meier method using the log-rank test and SPSS Statistics v.17 software.

Results: For the time being, 30 (55.6%) of 54 AA patients, 12 (70.6%) of 17 A/AOD patients and 34 (17.9%) of 190 GBM patients are followed up. The median survival and 2-, 3- and 5-year survival rates for grade III gliomas were 33 months, 71.5 ± 7.5%, 48.3 ± 7.5% and 39.1 ± 8.5%; for grade IV GBM - 14 months, 22.6 ± 3.2%, 11.2 ± 2.7%, 7.9 ± 2.9% respectively (p < 0.0001). The median survival of patients administered chemoradiotherapy for grade III glioma has not been attained yet, 3- and 5-year survival rates are 66.9 ± 11.1%; the median survival of patients receiving radiotherapy is 29 months, and 3- and 5-year survival rates are 36.8 ± 8.9% and 27.6 ± 8.8% respectively (p < 0.034). For GBM treated with chemoradiotherapy, the median survival and 2-, 3- and 5-year survival rates are 16 months, 25.2 ± 4.7%, 11.1 ± 3.9% and 7.4 ± 4.0%; with radiotherapy - 12 months, 18.3 ± 4.3%, 9.8 ± 3.3% and 8.4 ± 3.1% respectively (p < 0.035). The median survival for AO/AOD was higher then for AA and was 46 months and 29 months respectively (p < 0.069).

Conclusions: Postoperative conformal radio-(chemo)radiotherapy at a TTD of 54-60 Gy provides 2-, 3- and 5-year survival rates of 71.5 ± 7.5%, 48.3 ± 7.5%, 39.1 ± 8.5% and 22.6 ± 3.2%, 11.2 ± 2.7%, 7.9 ± 2.9% for grade III anaplastic glioma patients and grade IV GBM patients respectively, with median survival of 33 and 14 months respectively (p < 0.0001). Postoperative conformal radio-(chemo)radiotherapy improves treatment outcomes compared with radiotherapy for both anaplastic gliomas (p < 0.034) and GBM (p < 0.035).

EP-0999 Re-irradiation ± bevacizumab in recurrent or progressive HGG: retrospective analysis of 13 patients  
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Purpose/Objective: To review the safety and activity of radiotherapy with concurrent bevacizumab in recurrent malignant gliomas. Reasons to combine bevacizumab and RT include the ability of antiangiogenic agents to sensitize tumor endothelium to RT by depletion of VEGF and reduction of its pro-survival signaling. Our retrospective analysis provides additional data out of a very limited number of studies about safety and feasibility of conventional 3D-conformal re-irradiation in combination with bevacizumab as a salvage therapy for relapsed gliomas.

Materials and Methods: Patients with recurrence of malignant gliomas who failed after standard treatment ofd surgery, post operative radiotherapy ± Temozolomide received bevacizumab (10 mg/kg i.v.) every two weeks until tumor progression and hypofractionated (16 x 2.66 Gy). The interval between the two radiotherapy treatments was at least 4 months in our patients. The median physical doses of the first and second radiation course were 40-60 Gy and 39-60 Gy, respectively. The median cumulative biological equivalent doses (BED) were 215 Gy (α/β = 2 gy) and 100 Gy (α/β = 10 Gy); Median RT-volume was 143 cm³ and median cumulative RT-dose was 95 Gy. Results: 13 consecutive patients with recurrent malignant gliomas (6 GBM, 3 AAC, 3 LGG, 1 not applicable) received 2 cycles of bevacizumab prior to re-irradiation and underwent a repeat cranial MRI-scan for RT-planning. Patinets who respond or had SD proceed to radiation therapy in a 3D-conformal manner to 16 x 2.66 Gy: PTV included the contrast-enhancing GTV, the surragond oedema plus an additional margins of 2.5 cm. Critical structures like the optic chiasm were excluded and the dose to the re-irradiated target volume was restricted to a cumulative dose of 110 Gy. No Grade 3-4 acute toxicity developed, haematologic non-haematologic toxicities were never severe and none were transient. Until now 1 necrosis is seen in this cohort and all patients responded to therapy. The median PFS after re-irradiation is 4.9 months, the median OS after re-irradiation is 8.2 months. Conclusions: 3D-conformal re-irradiation ± bevacizumab is save with a good quality of life for progressive HGG patients. The large re-irradiation volumes were well tolerated with a low rate of one confirmed radiation necrosis in 13 patients. Pretreatment with radiochemotherapy (temozolomide) does not increase neurotoxicity. The PFS is comparable to more complex and less abundant high precision radiation techniques.

EP-1000 Use of MRI diffusion-weighted images for follow up assessment of radiosurgery effectiveness for meningiomas  
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Purpose/Objective: Meningiomas comprise one third of the primary brain tumor cases and occupy the second place (up to 20%) after gliomas concerning the extension among the tumors of the CNS. Surgical resection is regarded as choice method for meningioma treatment. However, in cases of complex localization and in case of placement near vital neurovascular structures, and also in case of partial resection or extended tumor growth, usage of stereotactic radiosurgery allows preventing of possible complications and reaching of radiosurgical control over the tumor.

Materials and Methods: 63 patients with meningiomas underwent treatment at MC’Cyber Clinic of Spizhenko’. Acquired results of 42 patients were analyzed. Among them 21 patient previously underwent surgery (1-3 operations), and 24 patients did not previously undergo surgery. Tumor volume was defined between 1.6 ml to 86.6 ml (on average 26.8ml). All the patients underwent course of stereotactic radiosurgery with the use of CyberKnife system for 1-5 fractions (on average 4.45) depending on the tumor volume. Radiation dose ranged from 1500 to 2800 cGy. All patients underwent standard assessments, as CT, MRI, and also magnetic-resonance imaging in regime of diffusion-weighed images (DWI) with definition of actual diffusion coefficient – ADC (Apparent Diffusion Coefficient). DWI and ADC diffusion-weighed images (DWI) with definition of actual diffusion coefficient in 27 (64.3%) of the patients. Hereinafter the conditions of those patients continued improving. At the same time, according to the literature data, without taking into account the ADC of MRI diffusion-weighed images, decrease of tumor (meningioma) after radiosurgery was observed only in 18%, and stabilization of the process was seen in 74% of the patients. ADC diffusion parameters in our patients with meningiomas before the start of treatment comprised 0.6-0.96 mm2/s. After radiosurgical treatment diffusion coefficient equaled 0.7-1.64 mm2/s.

Conclusions: Stereotactic radiosurgery with the use of CyberKnife is effective treatment method for brain meningiomas. Objective assessment of treatment results is possible only by means of standard radiological methods (CT, MRI), supplemented with special MRI modes (DWI) and ADC determination.

EP-1001
The influence of tumor-infiltrating lymphocytes on radiation necrosis in the patients with malignant astrocytoma
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Purpose/Objective: Radiation necrosis (RN) of the brain is an important problem for the patient with malignant astrocytoma, especially treated with high-dose RT. Individualized radiation dose delivery according to radiosensitivity may reduce the incidence of RN while keeping enough anti-tumor effect. And anti-tumor effect of irradiation was closely related to anti-tumor immune response of the host. The purpose of this study is to evaluate whether some parameters, which speculate the host’s immune strength, could predict the occurrence of RN at initial treatment.

Materials and Methods: Between 2006 and 2010, 64 patients with malignant astrocytoma were enrolled. All patients were treated with RT and concurrent temozolomide (TMZ) followed by adjuvant TMZ. Thirty-three patients received conventional RT and the other 31 received hypofractionated high-dose IMRT. In the IMRT group, three-layers of PTVs were contoured (PTV-1: enhanced lesion with 5mm margin, PTV-2: 15mm surrounding the PTV-1, PTV-3: FLAIR-high area) and different doses (68Gy for PTV-1, 40Gy for PTV2 and 32Gy for PTV-3) were delivered by 8 fractions. To estimate the immune strength of the patients, we used tumor-infiltrating lymphocytes (CD4+ helper T cells, CD8+ cytotoxic Tcells and FoxP3+ regulatory T cells (Treg) which suppresses the cytotoxic effect of CD8+ T cells) at first surgery, cerebrospinal fluid cells and proteins a week after first surgery. After treatment, MRI was performed with an interval of one or two months. We estimated the prognostic effect of these immunobiological parameters on overall survival (OS), progression-free survival(PFS) and RN-free survival (R NFS).

Results: High-dose hypofractionated IMRT induced RN more frequently than conventional RT (relative risk (RR) 3.44, p=0.0067) and tumor-infiltrating CD8+ T cells was also independent risk factor of RN (RR 3.10,p=0.0262). On the other hand, the infiltration of FoxP3+ Treg indicated lower risk of RN but not statistically significant. One-year R NFS for CD8 infiltration without FoxP3 and FoxP3 infiltration without CD8 were 50% and100%. Two-year R NFS were 0% and 86%, respectively (p=0.023). Tumor-infiltrating lymphocytes was not related to OS and PFS in the entire analysis. In the subgroup analysis of IMRT, the infiltration of CD8+ Tcells was higher risk factor of RN (RR 3.88, p=0.0041). In the conventional RT group, the infiltration of CD8+ Tcells was not the risk factor of RN but correlated with prolonged PFS (RR 0.21,p=0.0185).

Conclusions: Tumor-infiltrating CD8+ T cells was the prognostic factor of RN after RT, especially high-dose RT, but was not related to OS. The analysis of tumor-infiltrating lymphocytes might be useful to prevent the occurrence of RN.