P08.31 A PERIVASCULAR NICHE FOR PROGRESSION AND RESISTANCE IN Glioblastoma
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A distinct perivascular niche has been implicated in many aspects of tumor biology, particularly stemness, invasion, survival, and therapy resistance of cancer cells. But its overall importance for these factors remains obscure. By using multidimensional intravital imaging, that allows following individual glioblastoma cells in distinct brain microregions over long periods of time, we demonstrate that the perivascular niche plays a crucial role for many aspects of brain tumor progression. Glioblastoma stem-like cells (GBMSCs) colonized the perivascular niche in significant numbers, and used it as a route for effective brain invasion. This was partly explained by the fact that tumor microtubes (TMs), a novel cellular mechanism of glioblastoma progression and resistance (Oswald et al., Nature 2015), followed the perivascular room of the dense brain microcapillary network as a leading track. A subtraction of GBMSCs in a perivascular position showed long-term dormancy. Furthermore, location in the perivascular niche was associated with a high resistance against cytotoxic effects of radiotherapy, and with the ability of GBM-SCs to repair damage inflicted to their tumor microregion. Proficient NOTCH1 expression was required for successful perivascular niche colonization, and NOTCH1 deficiency reduced the radioresistance of perivascular tumor cells. Double-positivity for perivascular niche position and TM-formation was associated with the strongest primary resistance to therapies, and the strongest damage repair competence of an individual tumor cell. Taken together, these results provide the first direct evidence that a perivascular niche position is relevant for glioblastoma cells to exert central malignant traits, including those that have been associated with cancer stemness, that colonizing the subpopulation of glioma cells that colonizes the perivascular niche emerges as an important task for the development of novel therapies, since existing treatment modalities fall short of controlling these cells.

P08.32 TG02, AN ORAL CDK INHIBITOR, DEMONSTRATES ACTIVITY IN GLIOMA MODELS: EORTC BRAIN TUMOR GROUP CONDUCTS PHASE 1B STUDY (STEAM / EORTC 1608)
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Introduction TG02 is an orally-bioavailable, multi-kinase inhibitor. Its primary anti-tumor mechanism of action is through CDK9-dependent depletion (IC50 = 3mM) of oncoproteins such as Mcl-1 and MYC. TG02 also strongly inhibits CDK5 (IC50 = 4mM) MYC, Mcl-1 and CDK5 are frequently overexpressed in glioblastoma (up to 80%); concurrently or separately. In an in vitro panel of patient-derived glioma cell lines, TG02 demonstrated significant anti-tumor activity (IC50 range = 23–130 mM). At equimolar concentrations, TG02 was thus 200-2500 times more potent than temozolomide in the same panel. Clinical pharmacokinetics from a recently completed Phase 1b study in multiple myeloma demonstrate that TG02 exposures in humans are sufficient for achieving inhibitory concentrations required in the majority of the glioma cell lines tested. Preclinical studies in mice have demonstrated that TG02 is a good candidate for development in gliomas since TG02 is not a substrate of P-GP (in vitro) and concentrations in the brain are 2.4 times higher than in plasma.

Clinical Plan Based on the strong preclinical data for TG02, the EORTC Brain Tumor Group is currently conducting the STEAM 1608 study, a phase 1b dose-escalation and safety study of TG02 in combination with either hyperfractionated radiotherapy or temozolomide in elderly patients with glioblastoma. Patient allocation to treatment will be determined by MGMT promoter methylation status according to EANO guidelines for elderly patients with glioblastoma or anaplastic astrocytoma. We will report on the current preclinical stage of development of TG02 and update on the clinical trial to be conducted by the EORTC Brain Tumor Group.

P08.33 GLOSYTR (Glioblastoma Registry) OF THE AINO (ITALIAN ASSOCIATION OF NEURO-ONCOLOGY): FINAL ANALYSIS OF FACTORS INFLUENCING SURVIVAL IN Glioblastoma Patients Receiving the Nitrosourea FOTEMUSTINE AT FIRST PROGRESSION FOLLOWING CHEMORADIATION
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BACKGROUND: Patients with glioblastoma at first progression after standard radiotherapy and temozolomide (TMZ) often receive nitrosoureas, but factors influencing survival following these drugs are not well known. The aim of this retrospective study was to investigate the factors influencing progression-free survival (PFS) and overall survival (OS) in a cohort of 921 biopsy-proven glioblastoma patients, who received the nitrosourea fotemustine at first progression following chemoradiation.

PATIENTS AND METHODS: Survival data and information on 32 demographic, clinical, pathological, molecular and treatment modalities were obtained from 34 Italian Institutions between 2005 and 2014. Response and progression following fotemustine (FTM) were evaluated by RANO criteria. PFS and OS curves were drawn using the Kaplan-Meier method, and univariate and multivariate analysis with Cox regression model were performed.

RESULTS: Main patient characteristics before fotemustine were as follows: median age 57 (range, 18–80); males 587 (63.7%) and females 334 (36.3%); KPS 90–100 in 279 (30.3%) and 70–80 in 642 (69.7%); focal tumors in 661 (71.7%) and multifocal tumors in 260 (28.3%); reoperation in 236 (20.5%) with gross total resection in 78 and subtotal/partial in 158. MGMT status at first surgery was available in 453 (41%) patients with 201 methylated (44.4%) and 252 (55.6%) unmethylated. Fotemustine was given alone in 759 (82.4%) patients and in combination with bevacizumab in 162 (17.6%) PFS following fotemustine was 3.4 months. Multivariate analysis for PFS following fotemustine showed as independent prognostic factors: MGMT status HR 1.510, p=0.0001; KPS HR 1.220, p=0.0072; reoperation alone vs yes HR 1.292, p=0.0397; fotemustine alone vs fotemustine + bevacizumab HR 1.400, p=0.0002. OS following fotemustine was 6.2 months. Multivariate analysis for survival following fotemustine showed as independent prognostic factors: age (≥65 yrs vs <65 yrs; HR 1.217, p=0.0264; reoperation HR 1.445, p=0.0035; KPS HR 1.589, p=0.0001; fotemustine alone vs fotemustine + bevacizumab HR 1.221, p=0.0299.

CONCLUSIONS: MGMT methylation, high Karnofsky score and reoperation were strongly favorable prognostic factors for both PFS and OS. The association of fotemustine with bevacizumab was highly statistically significant for PFS but not for OS. Conversely, unilocularity of tumors was a favorable OS but not for PFS. The duration of adjuvant TMZ was not correlated with PFS and OS following fotemustine. On the other hand, MGMT methylation at first surgery can be used as a predictor of outcome following the nitrosourea fotemustine at recurrence.

P08.34 OVERALL SURVIVAL OF PRIMARY HIGH-GRADE GLIOMAS OF SUPRA- AND INFRAVENTORIAL MIDLINE STRUCTURES
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OBJECTIVE: In literature recent and robust data on the prognosis of patients with high grade gliomas (HGG) of WHO III and IV stratified by necrosis, molecular features and different treatment modalities are available. However, little is known about outcome of patients with HGG’s located in supra- and infratentorial midline structures like corpus callosum, basal ganglia and brainstem. In this study we evaluate overall survival (OS) and influencing factors of patients with de novo HGG specifically in these structures.

PATIENTS AND METHODS: In a single center retrospective analysis we screened our database for all adult patients with primary HGG in midline structures diagnosed by stereotactic biopsy (SB) between January 1996 and March 2015. We evaluated OS and analyzed factors (MGMT status, treatment after SB, age, gender, Karnofsky Score (KPS), localization) influencing OS using Whitney-Mann-U and Chi-square test as well as cox regression analysis. Results. 122 patients with HGG receiving stereotactic biopsy

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procedures [median age: 56.3 (22–82) years; median KPS: 80 (50–100%]. Histology revealed glioma WHO III in 31.1% and WHO IV in 68.9%. Lesions were localized in corpus callosum (30.0%), basal ganglia (18.5%), thalamus (9.0%), cerebellum (2.5%), cingula (0.8%), brainstem (18.0%). Median overall survival (mOS) was 6.3 months (95% CI, 3.6–9.0), for WHO III: 13.6 months (95% CI 5.6–21.6) and WHO IV: 4.9 months (95% CI, 3.2–6.6). Patients were treated as follows: no specific tumor therapy (33.1%, 2 WHO III and 15 WHO IV; mOS 11.1 months) or various Tumor specific therapy regimens (WHO III 34 (32.4%) patients mOS 15.5 months [95% CI 7.4–23.6]), [WHO IV] 65 (61.9%) patients mOS 10.6 months). In 6 patients (5.7%) the type of adjuvant therapy is unknown. MGMT promoter methylation was available for 46 tumor treated patients (19 WHO III, 28 WHO IV), mOS of patients with WHO IV tumors was significantly worse than WHO III (p<0.0001). Any tumor specific treatment improved survival significantly (p<0.005). Poor condition (KPS<70) and no tumor specific therapy were the significant factors for poor OS in multivariate analysis (adjusted HR 1.88 CI95% 1.03–3.92 p<0.04 and 11.09 CI95% 2.24–53.35 p<0.0001 respectively).

CONCLUSION: Overall survival de novo HGG’s especially of WHO IV located in midline structures is poor. Specific tumor treatment improves survival significantly. Treatment decision should be based on the patient’s clinical status for the best quality of life.

PO08.35 CLINICAL OUTCOMES FOR GLOBLASTOMA PATIENTS WITH SOLITARY, MULTIFOCAL AND MULTICENTRIC DISEASE
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INTRODUCTION: Based on imaging features, glioblastoma (GBM) can be classified as solitary, multifocal or multicentric. The incidence of GBM/multicentric GBM has been reported to range from 0.5 to 35%, and some studies have reported poorer survival in patients with multiple lesions. Two potential reasons have been proposed: (1) intrinsic differences in tumour biology; (2) failure to encompass the entire tumour within the radiotherapy planning tumour volume. To address this question we investigated clinical, imaging and genetic features in a cohort of GBM patients.

METHODS: Imaging, clinical, treatment, MGMT methylation and outcome data were collected retrospectively for 33 consecutive GBM patients treated in a single cancer centre between January 2011 and June 2012. Tumours were categorised as solitary, multifocal or multicentric by a consultant neuroradiologist.

RESULTS: 122 patients with GBM were identified. Median age was 60 and male:female ratio was 2:1:1. MGMT promoter status was unmethylated in 48% of tumours, methylated in 37% and unknown in 15%. Preoperative imaging modality was CT in 70% and MRI in 30% of patients. Overall, the proportion of patients with solitary, multifocal and multicentric tumours was 78%, 15.5% and 6.5% respectively, but in patients undergoing MR imaging these proportions were 60%, 26.5% and 13.5%. Gross total resection was performed in 65% of solitary compared with 42% of multifocal and 23% of multicentric cases, and radical chemoradiation was delivered to 50%, 38% and 38% of solitary, multifocal and 13% of multicentric cases. Overall, median survival was increased in solitary compared with multifocal/multicentric patients (9.9 vs. 6.7 months, p = 0.046). In patients receiving radical chemoradiation, however, there was no difference in overall survival between solitary and multifocal/multicentric patients (18.5 vs. 16.8 months, p = 0.57). MGMT promoter methylation was associated with increased survival in patients with solitary tumours (14.6 vs. 8.5 months, p = 0.014) but not multifocal/multicentric tumours (3.8 vs. 6.9 months, p = 0.3).

CONCLUSION: In this retrospective study the incidence of multifocal/multicentric GBM was in line with previous studies. Pre-operative CT imaging may underestimate the incidence of multifocal/multicentric disease. Our main finding was that multifocality or multicentricity did not affect survival in patients to whom radical chemo-radiotherapy could be delivered.

PO08.36 RADIORESISTANCE OF GLOBLASTOMA STEM-LIKE CELLS IS ASSOCIATED WITH DNA REPLICATION STRESS, WHICH IS A PROMISING THERAPEUTIC TARGET
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INTRODUCTION: The inevitability of tumour recurrence in glioblastoma (GBM) patients despite multi-modality treatment consisting of surgery, radiotherapy and chemotherapy, is reflected by a median survival of only 14 months. Tumour recurrence is thought to be driven by a small population of glioblastoma stem-like cells (GSCs) that are resistant to conventional therapies. DNA damage response (DDR) pathways have been shown to be up-regulated in GSCs and implicated in radioresistance and treatment failure. However the precise cause of enhanced DDR signalling in GSCs and the extent to which these signalling networks contribute to radioresistance remains elusive. The objectives of this study were to investigate the underlying cause of DDR upregulation and treatment resistance in GSCs with a view to identifying novel and promising therapeutic targets.

MATERIALS AND METHODS: A panel of primary patient derived GBM cell lines cultured under conditions to enrich for or deplete the tumour stem cell population (GSC vs bulk respectively) were utilised in order to investigate enhanced GSC DDR under basal conditions and in response to ionising radiation. Confirmation studies were also performed in cells sorted for the putative GSC marker CD133. The effects of a panel of small molecule DDR inhibitor agents on cell survival in GSC and bulk cells were quantified.

RESULTS: GSCs exhibited higher levels of total and activated DDR targets ATR, CHK1, ATM and PARP1 under basal conditions and were more resistant compared to paired bulk populations. This was not due to increased levels of reactive oxygen species (ROS). Instead, we show that RPA is significantly higher in replicating GSCs and confirm by DNA fibre assays that GSCs and CD133+ cells have increased numbers of stalled replication forks, fewer new origins and slower DNA replication compared to bulk or CD133- populations, demonstrating for the first time that replication stress (RS) is a hallmark of GSCs. We identify increased expression of long neural genes as a likely mechanism for RS and DNA double strand breaks (DSBs) in GSCs and show that their radioresistance is reversed by dual inhibition of key RS and DDR proteins ATR and PARP.

CONCLUSIONS: This study demonstrates the novel finding that replication stress is a hallmark of GSCs and resonates with recently published studies on glioblastoma cancer stem cells showing that RS preferentially induces DNA DSB in long neural genes. Taken together, we implicate RS as a driver of enhanced DDR in GSCs and identify novel therapeutics with potential to improve clinical outcomes by overcoming the radioresistance of GBM.

PO08.37 TUMOR ASSOCIATED M2 MACROPHAGE INFILTRATION IN GLOBLASTOMA
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INTRODUCTION: Anti-inflammatory phenotype (M2) macrophage is known to secrete various cytokines and promote tumor-growth. However, the role of M2 macrophage in glioblastoma is not clear yet.

METHODS: We evaluated the specimens resected from thirty-three GBM patients with the prognosis of patients with glioblastoma, who underwent surgery at Kobe University Hospital from November 2006 to December 2013. We investigated the infiltration of tumor-associated M2 macrophages by means of immunostaining for CD163, which is M2 macrophage markers. DNA methylation status was available for 46 tumor treated patients (18 WHO IV° 65 (61.9%) patients mOS 10.6 months). In 6 patients (5.7%) the type of adjuvant therapy is unknown. MGMT promoter methylation was associated with increased survival significantly (p=0.005). Poor condition (KPS<70) and no tumor specific therapy were the significant factors for poor OS in multivariate analysis (adjusted HR 1.88 CI95% 1.03–3.92 p<0.04 and 11.09 CI95% 2.24–53.35 p<0.0001 respectively).

CONCLUSION: Overall survival de novo HGG’s especially of WHO IV located in midline structures is poor. Specific tumor treatment improves survival significantly. Treatment decision should be based on the patient’s clinical status for the best quality of life.

PO08.38 IRRADIATION OF SUBVENTRICULAR ZONE IN GLOBLASTOMA: ITS IMPACT ON TUMOR PROGRESSION AND SURVIVAL
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INTRODUCTION: Irradiation of Subventricular Zone in Glioblastoma: Its Impact on Tumor Progression and Survival