PO08.31 A PERIVASCULAR NICHE FOR PROGRESSION AND RESISTANCE IN Glioblastoma

M. Osvald1, E. Jung1,2, S. Wei1,2, I. Jäger2, G. Solecki1–2, F. T. Kurz2, S. Heiland3, P. E. Huber1,4, W. Wick2,5, E. Winkler6 1Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, Germany; 2Clinical Cooperation Unit Neurol. Oncology, German Cancer Center (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany; 3Department of Neuroradiology, University Hospital Heidelberg, Heidelberg, Germany; 4Department of Radiation Oncology, University Hospital Heidelberg, Heidelberg, Germany; 5CCU Molecular and Radiation Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany.

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 (TM), a novel cellular mechanism of glioblastoma progression and resistance (Osvald 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 GBMScs 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, invasion, survival, and therapy resistance. The perivascular niche emerges as an important task for the development of novel therapies, since existing treatment modalities fall short of controlling these cells.

PO08.32 TG02, AN ORAL CDK INHIBITOR, DEMONSTRATES ACTIVITY IN GLIOMA MODELS: EORTC BRAIN TUMOR GROUP CONDUCTS PHASE 1B STUDY (STEAM / EORTC 1608)

T. Parron1, M. Weller2, T. M. Estok3, E. Le Rhun2 1Tragara Pharmaceuticals, Carlsbad, CA, United States; 2University Hospital Zurich, Zurich, Switzerland; 3Centre Hospitalier Regional Universitaire, Lille, France.

Introduction TG02 is an orally-bioavailable, multi-kinase inhibitor. Its primary anti-tumor mechanism of action is through CDK9-dependent depletion (IC50 = 3nM) of oncoproteins such as c-Myc and MYC. TG02 also strongly inhibits CDK5 (IC50 = 4nM; MYC, McI-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 nM). 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 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.
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%), striatum (2.5%), c.s., (0.8%), brainstem (18.8%), and 5.0% of cases remained unclassified. 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 (13.1%, 2 WHO III° and 15 WHO IV°; mOS 1.1 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=0.003). 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% 5.24–23.35 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.

P08.35 CLINICAL OUTCOMES FOR GLIOBLASTOMA PATIENTS WITH SOLITARY, MULTIFOCAL AND MULTICENTRIC DISEASE
Beatson Oncology Centre, Glasgow, United Kingdom.

INTRODUCTION: Based on imaging features, glioblastoma (GBM) can be categorized as solitary, multifocal or multicentric. The incidence of multifocal/multicentric GBM has been reported 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 all 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% solitary, 38% of multifocal and 13% of multicentric cases. Overall, median survival was increased in solitary compared with multifocal/multicentric tumours (9.9 vs. 6.7 months, p = 0.046). In patients receiving radical chemoradiation, however, there was no difference in overall survival benefit 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: 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 chemoradiotherapy could be delivered.

P08.36 RADIORESISTANCE OF GLOIOBLASTOMA STEM- LIKE CELLS IS ASSOCIATED WITH DNA REPLICATION STRESS, WHICH IS A PROMISING THERAPEUTIC TARGET
1University of Sunderland, Sunderland, United Kingdom, 2University of Glasgow, Glasgow, United Kingdom, 3University of Oxford, Oxford, United Kingdom, 4CR-UK Beatson Institute, Glasgow, United Kingdom.

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 ROS 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 replicates with recently published studies of normal progenitor 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.

P08.37 TUMOR ASSOCIATED M2 MACROPHAGE INFILTRATION IN GLIOBLASTOMA
Kobe University Graduate School of Medicine, Kobe, Japan.

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 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 cell marker. Also, we examined the association between the infiltration level of macrophages and CSF cytokines (IL-6, IL-10, sIL-2R) by Spearman's correlation coefficient by rank test. In addition, we examined the association between infiltration levels of M2 macrophages and prognosis by Log-rank test.

RESULTS: The mean infiltration rate of CD163-positive M2 cells in all glioblastoma specimens was 23.9% (3.7–64.7%). CD163-positive M2 macrophages tended to infiltrate in perivascular regions. Mean CSF concentration of IL-6, IL-10, sIL-2R in all glioblastoma patients were 30.5 pg/ml (0.8–292 pg/ml), <2 pg/ml (<2–5 pg/ml), and <50 pg/ml (<50~67 U/ml), respectively. The rate of CD163-positive M2 macrophage was significantly correlated with CSF IL-6 level (p<0.001). In relationship between CD163-positive M2 cells and progression free survival (PPS), glioblastomas with higher levels of CD163-positive M2 cells had significant shorter PPS in comparison with those with lower levels of CD163-positive M2 cells (p=0.046). However, there is no relationship between levels of CD163-positive M2 cells and OS. On the other hand, the patients with higher levels of CSF IL-6 had significant shorter PPS (p=0.015) than that with lower levels of CSF IL-6.

CONCLUSIONS: We observed high infiltration of CD163-positive M2 macrophages around the perivascular regions in glioblastoma. In addition, the infiltration levels of M2 macrophages correlated with IL-6 levels in CSF. The infiltration level of CD163-positive M2 macrophages may be associated with the prognosis of patients with glioblastoma.

P08.38 IRRADIATION OF SUBVENTRICULAR ZONE IN GLIOBLASTOMA: ITS IMPACT ON TUMOR PROGRESSION AND SURVIVAL
F. Setz, S. Hoca, S. Kamer, Y. Arıozık
Ege University, Izmir, Turkey.

Irradiation of Subventricular Zone in Glioblastoma: Its Impact on Tumor Progression and Survival.