retrospective analysis of treatment plans to assess the volume of SVZ irradiated using radical radiotherapy treatment.

METHODS: The T1-weighted contrast MRI scans of 100 consecutive Glioblastoma patients that had undergone maximal resection, were eligible for radical radiotherapy and chemotherapy were analysed. All patients were treated using IMRT (Intensity-modulated radiation therapy) delivered by the TomoTherapy system. The minimum distance between the gadolinium enhanced tumour resection cavity to the ipsilateral SVZ (which was defined as 5mm lateral to the lateral wall of the lateral ventricle) was measured. In a separate series of 22 patients, the overlap volume between the SVZ and the PTV (planning target volume) was measured. The PTV was generated by growing the GTV (gross tumour volume) isotropically by 30mm.

RESULTS: In the analysis of 100 Glioblastoma patients to determine if the SVZ overlapped with the PTV of the tumour, 97% of the patients received some incidental irradiation of their SVZ because it was included within the PTV. This relationship was observed regardless of tumour location. Volume analysis of the 22 patients showed that on average 33.5 % (8.5%-87.1%) of the patients' SVZ was irradiated.

CONCLUSIONS: Wide margins used in the treatment planning of Glioblastoma result in a high dose volume that almost always covers the SVZ adjacent to the tumour. However, if the entire ipsilateral SVZ is at risk of harbouring microscopic tumour residuum, it is not routinely covered by the high dose volume using conventional treatment planning protocols.

Given that this potential cancer stem cell niche may represent a therapeutically beneficially target, we have shown that there would be utility in a study that treated the entire ipsilateral SVZ within the high dose volume.

P08.24 CHARACTERIZATION OF GLIOMA CELL INVASION: TOWARDS NOVEL THERAPEUTIC TARGETS

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BACKGROUND: The aggressive potential of glioblastoma (GBM) is partially due to its highly invasive behaviour. Since invasive cells cannot be easily removed by surgery or irradiation, this tumour always recurs and is eventually lethal. Therefore it is crucial to elucidate the process of invasion in GBM to identify the key genes underlying the invasive capacity and may represent new therapeutic targets for GBM patients.

MATERIAL AND METHODS: The invasive behaviour of patient-derived glioma cell lines grown as neurospheres was characterized using different in vitro invasion assays. To target candidate genes responsible for invasion a whole genome library shRNA screen was performed. Furthermore, the in vivo invasive behaviour of glioma cell lines was investigated in orthotopic xenografts in mice and in slice cultures of adult mouse brain. Immunohistochemical analyses of xenografts were assessed at different time points of tumour development. Immunofluorescent staining against proliferating and invasive cells in xenografts were performed.

RESULTS: Glioma cell lines grown as 3D neurospheres displayed variable degrees of invasion in vitro and in vivo, and their invasive potential in vivo was reflected in their in vitro behaviour. E.g. the cell line, which developed a circumscribed, angiogenic phenotype in vivo showed the least invasion, while the cell lines with invasive phenotypes in vivo also displayed highest invasive scores in vitro. In addition, different developmental steps of tumour invasion were identified in orthotopic xenografts and the ratio of proliferating versus invasive cells was determined. Preliminary data on invasion-essential genes identified from shRNA interference screen will be presented.

CONCLUSION: The in vitro invasive behaviour of patient-derived glioma cell lines mirrors their in vivo invasive characteristics. Our data show that cells can proliferate and migrate at the same time, indicating a 'go-and-grow' process. Whole genome shRNA interference screen is a powerful tool to identify novel candidates to target the invasion process in GBM.

P08.25 DE NOVO TRANSCRIPTOME ASSEMBLY FINDS NOVEL GLIOBLASTOMA SUBTYPE-SPECIFIC TRANSCRIPTS

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Recent publications have proposed that adult glioblastoma (GBM) can be classified into four methylation subtypes; however, the underlying biological differences between these subtypes remains unclear. Our project has produced a rich set of genomic data (WGBS, strand-specific RNAseq, WES, histone mark ChIPseq) for 48 adult GBMs that is now being analysed to further characterise these four subtypes. We have established a transcriptome assembly pipeline using StringTie, followed by differential gene expression analysis with limma to identify novel subtype-specific transcripts. The pipeline finds 14209 novel transcripts in 6631 genes, 90% of which have not been previously annotated. The majority of these transcripts appear to be non-coding, with a subset predicted as containing protein domains. The subtype-specific novel transcripts identified by limma are supported by subtype-specific enrichment of histone modifications. A selection of candidate novel transcripts will be further characterised in silico and, potentially, in cell line models.

P08.26 VENOUS THROMBOEMBOLISM AND SURVIVAL IN PATIENTS WITH GLIOBLASTOMA MULTIFORME

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INTRODUCTION: The most aggressive form of primary brain tumors is glioblastoma multiforme (GBM). Despite the improvement of overall survival by combined treatment with radiotherapy and temozolomide, median survival remains only 14.6 months. During their short life time patients suffer from comorbidity such as venous thromboembolism (VTE). The prevalence of VTE in patients with GBM varies between 7.2 and 30 percent and is high as compared to patients with other malignancies. Previous studies showed no significant difference in survival between GBM patients with or without VTE. In this study we investigated the prevalence of VTE and correlation with survival in a large cohort of patients with GBM.

METHODS: This study included 263 patients with histological proven GBM treated in the Elisabeth-Tweesteden Hospital, The Netherlands, between 1th of January 2009 and 31th of December 2011. We investigated retrospectively the prevalence rate of symptomatic deep venous thrombosis (DVT) and pulmonary embolism (PE) diagnosed by ultrasound or CT-scan. Furthermore, we investigated the type of surgery and adjuvant treatment. The Kaplan-Meier method was used to estimate overall survival (OS).

RESULTS: One hundred forty-six patients received chemoradiation (CRT). Fifty-two patients were treated with radiotherapy only. The median follow-up time was 69 months (49–109 months). The median OS after the diagnosis of GBM was 9 months. In patient that received CRT, the OS was 16 months. Twenty-eight patients (10.65%) developed a VTE of which 12 DVT's (4.6%) and 17 PE's (6.5%). One patient was diagnosed with both DVT and PE. Among patients whom developed VTE 28.6% underwent biopsy only, 14.3% partial resection and 57.1% gross total resection. Out of the patients without VTE 36.2% underwent biopsy only, 10.2% partial resection and 53.6% gross total resection. Eighty-two point one percent of the VTE patients underwent CRT vs. 52.3% of the patients without VTE. The median OS in the VTE group was 13 months vs. 9 months in the non-VTE group, although the difference was not significant (p 0.203). The survival after the different types of surgery was significantly different ($p < 0.05$) with a median OS after biopsy of 4 months, 9 months after subtotal resection and 15 months after gross total resection. The dissimilarity in OS with respect to surgery was present in both VTE and non-VTE group and was not significantly different between the two groups.

CONCLUSION: The prevalence of venous thromboembolism in patients with glioblastoma multiforme is high. In our study population the median OS was 9 months and the OS differed significantly according to the type of surgery patients underwent. Although not significant, we found a trend towards better median overall survival in GBM patients that developed a VTE during the course of their disease versus no VTE.

P08.27 THE ROLE OF SUPRAMARGINAL RESECTION FOR SINGLE LARGE BRAIN METASTASES: FEASIBILITY, MORBIDITY AND LOCAL CONTROL EVALUATION

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PURPOSE: The role of surgical resection for single, large brain metastases (≥2.1 cm) is not clearly defined yet. Various surgical strategies have been adopted with the aim to achieve a resection as wide as possible keeping functional neurological integrity. Aim of the study was to evaluate the safety and feasibility of supramarginal resection in terms of post-operative morbid-

ity and brain tumor local control (LC). Brain distant progression (BDP) and overall survival (OS) were evaluated as well.

METHODS AND MATHERIALS: This retrospective study includes 69 patients with single large brain metastases. All underwent supramarginal resection with dural attachment radicalization plus hypofractionated stereotactic radiosurgery (HSRS). Clinical outcome was evaluated at thirty-days postoperative and by MRI performed every 3 months. Local progression was defined as an increase on MRI of enhancing in the treated area, and distant failure as occurrence of new brain metastases or leptomeningeal enhancement.

RESULTS: Clinical remission of symptoms was obtained in 90.5%. No patients had new neurological deficit or worsening of preoperative functional status. No major complications or CSF leakage occurred. No residual tumor was detected on postoperative-MRI. The median follow-up was 24 months (range 4–33 months). The 1-2-year LC was 100%. Twenty-four (29%) patients had new BDP and 75% also extracranial progression. The median, 1-2-year OS was 24 months, 91.3% and 73%. At the last observation time, 15 (21.7%) patients are dead and 54 (78.3%) alive.

CONCLUSION: Supramaginal resection along with dural attachment radicalization is a safe and effective approach for surgical treatment of single large brain metastases.

P08.28 ROLE OF SURGICAL RESECTION IN RECURRENT GLIOBLASTOMA: PROGNOSTIC FACTORS AND OUTCOME EVALUATION

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PURPOSE: The prognosis for newly diagnosed glioblastoma (GBM) has not yet undergone significantly improvements with a median survival time of 15–18 months. Local recurrence is the main reason of failure. Different treatment strategies are considered including a further surgery, second line chemotherapy, re-irradiation or combined treatment with different results recorded. The main question is which patients could have benefit from a second surgery, to date not well defined. The aim of the study was to evaluate the role of entity of surgical resection and peri-operative morbidity and mortality in patients with recurrent GBM. Progression free survival (PFS), overall survival (OS) and relevant prognostic factors were evaluated as well.

METHODS AND MATERIALS: From September 2008 to December 2014, 64 patients with recurrent GBM underwent second resection. Inclusion criteria were: KPS ≥70, age < 70, interval time from previous surgery longer than 6 months, no multifocal disease, tumor amenable of maximally surgical resection respecting functional boudaries. Adjuvant treatment consisted in RT (45 Gy in 15 fractions) and/or chemotherapy (CHT) consisted of TMZ or Fotoemustine. The entity of surgical excision was evaluated by the MRI acquired within 48–72 hours post-operatively. Clinical outcome was evaluated by neurological examination at admission and discharge and brain MRI performed one month after treatment and then every 3 months. Thirty-days postoperative morbidity and mortality were evaluated. Local progression was defined as radiographic increase of the enhancing abnormality in the treated area on serial MR imaging, and distant failure as the presence of new brain enhancing lesions outside the treated area.

RESULTS: The median interval time from the initial diagnosis to the recurrence was 17 months (range 8–63 months) and all 64 patients received surgical resection. Gross total resection (GTR) was obtained in 48 (75%) patients and subtotal (SR) in 16 (25%). Residual tumor volume (RTV) was present in 24 (37.5%) patients. Adjuvant treatments were performed in 51 (69.7%) patients: RT alone in 8 (15.7%), CHT alone in 15 (29.4%), concomitant and/or adjuvant radiochemotherapy in 28 (54.9%). At a median follow up time of 6.7 months (range 2.2–25 months) 24 (37.5%) patients were alive and 40 (62.5 %) were dead. The median, 6 and 12 months PFS were 6.8 months, 57.7% and 32.4%, and the median, 6 and 12 months OS were 10.4 months, 75.4%, and 21.6% respectively. On univariate and multivariate analysis factors conditioning survival were age KPS ($p<0.001$), the entity of surgical resection ($p=0.001$) and adjuvant treatments (0.04) performed. No major perioperative morbidity or mortality occurred.

CONCLUSIONS: surgical resection is a safe, feasible and effective treatment in selected patients with an adequate interval time from primary treatment, good KPS and age < 60. Prognostically pivotal is RTV.

P08.29 HISTOLOGIC STUDY OF MICROCIRCULATION PATTERNS IN HUMAN GLIOBLASTOMA

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INTRODUCTION: Glioblastoma is one of the most angiogenic human tumors and characterized by microvascular proliferations. A better understanding of glioblastoma vasculature is needed to optimize anti-angiogenic therapy that has shown a promising but incomplet efficacy. Vasculogenic mimicry (VM) is known as non-endothelial tumor cell-lined microvascular channels in aggressive tumors, which may function as blood supply networks.

MATERIALS AND METHODS: For observation of microcirculation patterns in glioblastoma, we examined 80 clinical glioblastoma samples and 20 glioma xenograft samples by immunohistochemistry (CD34 and periodic acid-Schiff) and immunofluorescence (CD34 and GFAP) dual staining.

RESULTS: We found there were four types of microcirculation pattern in human glioblastomas: endothelium dependent vessels, tumor cell dependent vessels, extracellular matrix (ECM) dependent vessels, and mosaic vessels. We confirmed subsequently that these four types of blood supply sources for tumors also exist in xenograft model glioblastomas.

CONCLUSIONS: Here, we described and sorted the microcirculation pattern in human glioblastomas systematically according to histologic evidences and open a new perspective for the anti-vascular treatment strategy.

P08.30 EXTENDED TEMOZOLOMIDE FOR NEWLY DIAGNOSED GLIOBLASTOMA: AN ANALYSIS OF THE GERMAN GLIOMA NETWORK

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BACKGROUND: The standard of care for newly diagnosed glioblastoma patients includes surgery followed by radiotherapy plus concurrent and maintenance chemotherapy with temozolomide (TMZ/RT→TMZ) for up to 6 cycles. The impact of prolonged TMZ treatment beyond 6 months remains controversial.

METHODS: The German Glioma Network (GGN) cohort was screened for newly diagnosed glioblastoma patients who received TMZ/RT->TMZ and completed at least 6 cycles of maintenance chemotherapy. Patients were divided in two groups: chemotherapy for 6 cycles (group A) compared to prolonged treatment regimens (group B). Associations of clinical patient characteristics, *O6-methylguanine DNA methyltransferase (MGMT)* promoter methylation status and residual tumor burden determined by MRI after 6 cycles of TMZ with progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method. Multivariate analyses using the Cox proportional hazards model were performed to assess associations of prolonged TMZ use with outcome.

RESULTS: 144 patients were identified: 74 patients received at least 7 maintenance TMZ cycles (median 11, range 7–20). Median PFS (group A 22.0 months, 95% confidence interval (CI) 17.8–26.2, versus group B 18.3 months, 95% CI 14.7–21.9, $p = 0.992$) and OS (group A 38.0 months, 95% CI 32.5–43.5, versus group B 31.0 months, 95% CI 26.2–35.8, $p = 0.848$) did not differ between groups. There was no significant association of prolonged maintenance TMZ chemotherapy with PFS (Hazard ratio (HR) = 0.8, 95% CI 0.3–1.8, $p = 0.549$) or OS (HR = 1.1, 95% CI 0.5–2.5, $p = 0.892$) adjusted for relevant clinical characteristics (age, extent of resection, Karnofsky Performance Score, presence of residual tumor) and *MGMT* promoter methylation status. In multivariate models relevant hazard ratios for residual tumor (PFS: HR = 3.6, $p = 0.003$; OS: HR = 3.8, $p = 0.003$) were observed. *MGMT* promoter methylation status was associated with HR = 0.6 ($p = 0.250$) for PFS and HR = 0.5 ($p = 0.091$) for OS.

CONCLUSION: These data do not support the practice of prolonging maintenance TMZ chemotherapy beyond 6 cycles, irrespective of *MGMT* promoter methylation status or residual tumor after 6 cycles.