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# 2013 WFNO-SNO Abstracts

## OMICS AND PROGNSTIC MARKERS

### OM-001. CONSIDERATION OF RELATIONSHIPS BETWEEN GENETIC ABNORMALITY AND CLINICAL PICTURE INGLIOMA WITHOUT +7 AND −1p/19q

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Although histopathological diagnosis is essential in decision of therapeutic strategy for gliomas, sometimes the tumors diagnosed in one histological entity show thoroughly different clinical courses. This phenomenon is believed to be due primarily to the presence of the genetic subgroup. In fact, relationship between treatment response and certain genetic characteristics is indicated (e.g. better chemosensitivity in glioma with losses of 1p/19q (−1p/19q)). It is highly likely that genetic classification of glioma is useful to select the adjuvant treatment. Additionally, gain of 7q (+7q) and −1p/19q are early events in 2 distinct tumor lineages, astrocytic tumors and oligodendroglial tumors, respectively, and these tumors obtain additional genetic aberration (−9p, 10q) with tumor progression. On the other hand, concerning the tumors without +7q or −1p/19q, little is known about clinically important genetic aberration. Therefore the study on such tumors could provide useful information for the prognosis prediction and the determination of treatment strategy. METHODS: We selected 39 cases of gliomas without +7q or −1p/19q from 200 adult supratentorial glioma cases surgically treated and analyzed chromosomal DNA copy number aberrations (CNAs) by comparative genomic hybridization (CGH) from 2005 to 2012. We correlated clinical features of these tumors with histological characteristics, CNAs and IDH1 status. RESULTS: The clinical course of gliomas without +7q or −1p/19q was not correlated with additional genetic aberration of -9p or 10q, which have been known as genetic markers for poor prognosis, and absence of +7q or −1p/19q was maintained at the time of recurrence. The tumors without +7q or −1p/19q showed relatively favorable prognosis although mutation of IDH1 was infrequent in these tumors (35.8 %). CONCLUSION: The gliomas without +7q or −1p/19q have clinical features distinct from the +7q and −1p/19q gliomas. Prognostic markers for each subgroups could help establish therapeutic strategy against the tumor.

### OM-002. MOLECULAR CHARACTERIZATION OF 11p-DELETED DIFFUSE LOW-GRADE GLIOMAS

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Diffuse low grade gliomas (LGG) form a heterogeneous subgroup of glial tumors. Over the last years, several genetic and genomic alterations, with clinical and biological relevancies, have been identified in LGG: (i) 1p/19q co-deletion and (ii) recurrent mutations in IDH1/IDH2, ATRX, TP53, CIC and FUBP1 genes. In a previous study, we have identified a subgroup of non 1p/19q-codeleted LGG exhibiting 11p deletion, astrocytic phenotype and poor prognosis. In order to better characterize 11p-deleted LGG, we have performed: (i) whole-exome sequencing in 5 samples, (ii) gene expression profiling and copy number profiling in 44 LGG. We have also analyzed epigenomic profiles of 11p-deleted LGG using The Cancer Genome Atlas (TCGA) data available publicly. LGG exhibiting11p loss were: (i) IDH mutated/CIC intact/FUBP1 intact in 100% of cases and (ii) ATRX mutated/TP53 mutated in 75% of cases. Interestingly, two original genes involved in telomerase and in chromatin remodeling pathways were found mutated in 12% and 6% of cases respectively. Transcriptomic profiling showed overexpression of inflammatory genes in 11p deleted LGG. Finally, TCGA methylomic data highlighted that 11p deleted LGG are hypermethylated. Our study participates to decipher LGG revealing that 11p-deleted LGG form a homogeneous histo-molecular subgroup of tumors with inflammatory component. In addition, our study identified new gene mutations potentially involved in oncogenesis of this tumor type.

### OM-003. MOLECULAR FEATURES OF ADULT PATIENTS WITH GANGLIOGLIOMAS

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BACKGROUND: Gangliogliomas represent less than 1% of primary brain tumors in adults. Little is known regarding molecular characteristics and their potential impact on patient outcomes. METHODS: In this IRB approved retrospective study, our neuro-oncology longitudinal database was screened for patients with gangliogliomas from 1992-2012. 69 adult patients (age > 18) were identified, of whom 29 patients had 32 tissue samples eligible for analysis. DNA was extracted from archival tumors and hot spot mutation testing was performed using targeted ultradeep exome sequencing of approximately 200 cancer related genes in the research environment; copy number, loss of heterozygosity and intratumoral heterogeneity were assessed. RESULTS: 24 (83%) patients presented with low grade gangliogliomas, 5 (17%) presented with high grade gangliogliomas. The median age at diagnosis was 27 years (18-65). The median KPS at presentation was 100 (70-100). Patients underwent gross total resection (16, 55%), subtotal resection (11, 38%), or biopsy (2, 7%). The median overall survival for all patients was 4.1 years. 6 of the patients with low grade gangliogliomas had malignant transformation to a higher grade. The median overall survival for those patients was 1.1 years. Tissue submitted for analysis represented 17 low grade and 9 high grade samples from unique patients. In 3 patients, tissue was submitted from an initial low grade and then a recurrent high grade sample. Complete molecular profiling data will be presented. CONCLUSIONS: While gangliogliomas have an excellent prognosis, some patients have more aggressive tumors especially those undergoing malignant transformation. Molecular characterization of low and high grade gangliogliomas will provide additional insight into the biologic behavior of these tumors.

### OM-004. CLONAL EXPANSIONS AND EVOLVING SUBPOPULATIONS IN GLIOBLASTOMA MULTIFORME

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MOTIVATION: Cancers are thought to arise from a single cell that has acquired a mutation that provides a survival benefit. The clonal evolution model indicates that cells with initiating mutations proceed to acquire additional mutations. Growth advantageous mutations cause the expansion of cancer cell subclones, resulting in tumors that consist of multiple, genetically distinct subpopulations. This genetic heterogeneity is thought to increase a tumor's survival chances when confronted with therapies by providing a diverse repertoire of phenotypic responses. A systematic approach to characterize tumor cell subpopulation diversity may facilitate improved understanding of the genetic determinants of recurrence and resistance to therapy. RESULTS: We present a bioinformatic method, ExPANdS, which estimates the proportion of cells in a tumor that harbor a specific mutation. By modeling cellular frequencies as probability distributions, ExPANdS predicts mutations that accumulate in a cell prior to its clonal expansion. Applied upon a cohort of 45 Glioblastoma tumor-specimens available from TCGA, the approach predicted

the extent of subclonal diversity, the proportion of subpopulations in individual tumors, and subpopulation specific mutations. We found that mutations within DNA-repair genes occur early in tumor development and are accompanied by an increased number of subsequent mutations. We also predicted the subpopulation composition of matched primary and recurrent Glioblastoma and used this information to identify SPs that contribute to recurrence in primary Glioblastoma. CONCLUSION: Our results provide a predicted sequence for mutational events in gliomagenesis and present a model for the clonal evolution of individual Glioblastoma tumors prior to and following treatment. This approach may lead to the identification of mutations that provide resistant tumor cell populations with the ability to survive therapy.

## OM-005. NOVEL GENES AND GENE SIGNATURE IN PROGNOSTICATION OF GLIOBLASTOMA: POTENTIAL TARGETS AND PATHWAY IDENTIFICATION

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BACKGROUND: Recent research on glioblastoma (GBM) has focused on deducing gene signatures predicting prognosis. Further, a number of candidate genes are explored to have role in pathogenesis or prognosis. AIMS: We evaluated mRNA expression of selected genes and correlated with outcome to arrive at a prognostic gene signature. Further we explored some of the genes in the signature for their role in prognosis. A comparison of the published gene signatures was performed. METHODS: Patients with GBM were prospectively recruited and underwent uniform standard therapy. qRT-PCR was used to study expression profile of 175 genes in GBM tissue. A 14 gene signature predicting prognosis was derived using a supervised principal component analysis and validated with TCGA data. Gene Ontology and KEGG pathway analysis was carried out among patients from TCGA cohort. The signature was compared with other published gene signatures. Some of the genes were further explored for their role in glioma pathogenesis. RESULTS: A weighted gene score derived from the 14 gene signature was found to be an independent predictor of survival in multivariate analysis in the present cohort (HR = 2.507; B= 0.919; $p < 0.001$) and in TCGA cohort. The standardized WG score classified patients into low and high risk predicting survival. Pathway analysis using the most differentially regulated genes between groups revealed association of activated inflammatory/immune response pathways and mesenchymal subtype in the high risk group. SOD2, OLFM1, TOP2A were further explored. SOD2 gene predicted survival independently in GBM and showed significant grade specific variation. We also studied the role of TOP2A gene expression in GBM and its influence of temozolomide therapy in GBM. CONCLUSION: We have identified a 14 gene expression signature that can predict survival in GBM patients, which also revealed a link to immune response pathways. Exploration of individual genes reveals potential targets for further studies.

## OM-006. GENETIC MUTATIONS IN EPIGENOMICALLY-DEFINED PROMOTERS AND ENHANCERSIN GLIOBLASTOMA

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Cis-regulatory element (cRE) mutations underlie hereditary cancers and may contribute significantly to sporadic cancer, but cancer genetics research to date has primarily focused on the protein coding exome. Non-coding mutations can contribute to tumorigenesis by altering transcription factor (TF) binding and the expression level of their target gene(s). Determining which cRE mutations are functional and might be drivers of a malignant phenotype in part depends on the cell-type specific chromatin state and the presence of relevant transcription factors. Only a fraction of cREs in the genome are active in a given cell type, and many show little conservation between species, making their identification difficult using comparative genomics alone. Epigenome mapping of just three histone modifications using ChIP-seq allows for the identification of a majority of active cREs in any accessible tissue type. We generated these "chromatin state" maps from primary Glioblastoma multiforme (GBM) and adult normal brain tissues, and intersected publicly available GBM whole genome and transcriptome sequencing data to identify novel mutations in cREs. We hypothesize that cRE mutations contribute substantially to tumorigenesis in Glioblastoma multiforme (GBM). Highly recurrent mutations in the promoter of TERT have been recently discovered in GBM, and we assessed further its allelic affect on the local chromatin state. We have discovered and validated novel cRE mutations under apparent positive selection, and computationally predicted the effect of each cRE mutation on TF binding. cREs that are recurrently mutated were evaluated for their impact on gene expression in luciferase reporter assays. This study provides an experimental and computational framework to identify driver cRE mutations that is widely applicable to most other tumor types.

## OM-007. INTEGRATIVE EPIGENOMICS TO IDENTIFY DRIVER EPIMUTATIONS IN GBM

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Glioblastomas (GBMs) accumulate epigenetic alterations in DNA methylation that result in aberrant gene expression and influence tumorigenesis, progression, and response to therapy. Cancer-specific changes in the post-translational modifications of histones also regulate gene expression states and are targetable with epigenetic-based therapies, but are unexplored in GBM to date. For both DNA methylation and histone modification epimutations however, separating driver from passenger events remains challenging. We integrated DNA methylome data with sample-matched "activity states" of promoters and enhancers, defined by the combinatorial pattern of just three histone modifications using ChIP-Seq. We compared H3K27Ac signal between our GBM samples and normal brain samples generated from the Roadmap Epigenomic Mapping Consortium (REMC), to identify promoters and enhancers that are active, poised or inactive. Recurrently hypermethylated loci were strongly enriched at both promoters and distal enhancers active in normal brain, significantly more than non-recurrent hypermethylation, highlighting their potential to silence gene expression. While most gains and losses of active promoter/enhancer states in GBM were independent of DNA methylation changes, 12.6% of those lost in GBM were also hypermethylated, a much greater proportion than expected by chance ($P < 2.2e-16$). We hypothesize that combinatorial epimutations involving both recurrent DNA hypermethylation and loss of "active" histone modifications are more likely to be true inactivation events compared to hypermethylation alone, which can occur passively at transcriptionally inactive genes. To this end, we found 274 promoters and 36 enhancers that are recurrently hypermethylated across GBM samples and which also display recurrent GBM-specific loss of H3K27Ac. 122 genes associated with these combinatorial epimutations display significantly decreased expression across 400 GBMs profiled in the TCGA project. Our approach, which integrates multiple epigenomic data types from primary tumors, has the potential to enrich for candidate driver epimutations, which have been challenging to identify against the abundant passenger epigenetic alterations in GBM.

## OM-008. QUANTIFICATION OF TUMOR-SPECIFIC DNA AS A PREDICT MARKER FOR MENINGIOMAS

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PURPOSE: This approach has not been studied in meningiomas. Increased levels of tumor-specific DNA have been described in various malignancies as a diagnostic and prognostic biomarker. This approach has rarely been studied in meningiomas. We analyzed the significance of Topoisomerase IIα (TOP2A) in patients with meningiomas. METHODS: Paired tumor-serum samples from 27 primary benign meningiomas were analyzed. 22 non-cancer individuals serum were used as control. DNA was extracted from tumor and serum samples. The median interval between surgery and serum sampling was 1.5 months. The level of TOP2A was studied by real-time PCR. RESULTS: High-level of TOP2A was also detected in tumor and serum samples. The rate of serum detection of these biomarkers was 52%, respectively, with specificity around 100%. Statistically significant tumor-serum concordance was found for the TOP2A in patients ($r = 0.87$, $p < 0.01$). None of the control serum showed aberrant TOP2A. TOP2A was higher level in patients with tumor recurrence ($p < 0.01$). CONCLUSIONS: Quantification of tumor-specific DNA by real-time PCR may be a simple tool for detection of meningiomas with a potential to clinical applicability together with other current methods used for monitoring the disease.

### OM-009. CONTROL OF SOCS1 AND SOCS3 METHYLATION, AND RELATIONSHIP WITH MGMT AS EFFECTIVENESS FACTOR OF TREATMENT IN GLIOBLASTOMA

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INTRODUCTION: Glioblastoma (GBM) is the most frequent and aggressive brain tumor in adults. Standard treatment is surgery followed by radiation and chemotherapy in selected patients; however, has a high recurrence rate. $O^6$-methylguanine–DNA methyltransferase (MGMT) promoter methylation is a predictive factor of response to Temozolomide. Suppressors of cytokine signaling 1 (SOCS1) and SOCS3 genes seem to be deregulated and cause radioresistance in GBM. No studies has been done about methylation and radioresistance in this tumor, so we hypothesize that methylation of SOC1 and SOCS3 and relationship with MGMT could be good prognostic and predictive factors for chemoradiation response in GBM. METHODS: Methylation status of SOCS1, SOCS3 and MGMT was analyzed by methylation-specific PCR in 100 GBM tissues, and paired with control samples. Some examples were also sequenced. Subsequently, results were combined with a clinical database and analyzed with SPSS software. RESULTS: All GBM tissues were tested for methylation of SOCS1, SOCS3 and MGMT. We observe high levels of SOCS1 gene methylation (>50%) not so in SOCS3 (<15%) gene. MGMT promoter methylation status was founded in 52 % of samples. Patients with methylated SOCS1 gene, unmethylated SOCS3 gene and unmethylated MGMT gene had worse response to chemoradiation therapy and worse overall survival. CONCLUSIONS: Epigenetic silencing of SOCS1 and SOCS3 expression could be good biomarkers for radiation response. Combination of SOC1, SOCS3 and MGMT seems to be good prognostic and predictive factors for chemoradiation in the GBM treatment setting.

### OM-010. LOSS OF CIC AND FUBP1 EXPRESSIONS ARE POTENTIAL MARKERS OF SHORTER TIME TO RECURRENCE IN OLIGODENDROGLIAL TUMORS

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Combined deletion of chromosomes 1p and 19q is a prognostic marker in oligodendroglial tumors (OTs). Recent studies in OTs have unveiled recurrent mutations of CIC and FUBP1 that are located on 19q13 and 1p31, respectively. However, the impact of CIC and FUBP1 mutations on their protein expressions has not been examined. The aims of this study were to correlate the expression patterns of CIC and FUBP1 with their mutation profiles and to evaluate the clinical relevance of these molecular markers in 55 OTs diagnosed in 47 adult patients. Using direct sequencing, somatic mutations of CIC and FUBP1 were identified in 46.8% (22/47) and 15.6% (7/45) of OTs, respectively. Immunohistochemical analysis revealed loss of CIC or FUBP1 expression in 36.4% (20/55) and 16.4% (9/55) of OTs examined. Somatic mutation was significantly associated with absent protein expression for both genes (CIC, $P = 0.01$; FUBP1, $P = 0.00001$). Four tumors with undetectable CIC mutations exhibited absent CIC expression, suggesting that CIC inactivation could be mediated by mechanisms other than mutations and genomic loss. Univariate survival analysis revealed that 1p/19q codeletion was significantly associated with overall survival ($P = 0.05$). Loss of CIC expression was significantly correlated with shorter progression-free survival ($P = 0.03$), whereas CIC alteration (mutation or null expression) with worse overall survival ($P = 0.05$). Absent FUBP1 expression was linked with unfavorable progression-free survival ($P = 0.02$) and overall survival ($P = 0.01$). In 16 tumors with 1p/19q-codeletion, CIC mutation was associated with unfavorable survival ($P = 0.01$). There was a correlation between lack of CIC or FUBP1 expression and poor progression-free survival ($P = 0.004$; $P = 0.0003$). No molecular markers showed association with survival in OTs lacking 1p/19q codeletion. We conclude that absent CIC and FUBP1 expression are potential markers of shorter time to recurrence and CIC mutation a potential marker of worse prognosis, especially in tumors carrying 1p/19q codeletion.

### OM-011. HYPERPOLARIZED [1 -$^{13}$C] α-KETOGLUTARATE: A NOVEL DNP PROBE FOR NON-INVASIVE ASSESSMENT OF IDH1 MUTATIONAL STATUS IN GLIOMA

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INTRODUCTION: Given the prevalence of isocitrate dehydrogenase 1 (IDH1) mutations in gliomas and upgraded glioblastomas (GBM), mutant IDH1 inhibitors are being developed as potential new therapies, and non-invasive methods to monitor IDH1 status in vivo are needed. Because mutant IDH1 converts α-ketoglutarate (αKG) into 2-hydroxyglutarate (2HG), imaging methods to detect IDH1 status have focused to date on detecting 2HG using $^1$H magnetic resonance spectroscopy (MRS). Our goal was to validate $^{13}$C MRS of hyperpolarized (HP) αKG as a complementary method probing IDH1 enzyme activity in vivo. METHODS: U87 GBM cells were transduced with a vector coding for wild-type (U87IDHwt) or mutant and wild-type IDH1 (U87IDHmut). Orthotopic tumors were implanted in the right putamen of rats. After polarization and dissolution, HP αKG was injected intravenously. 2D CSI dynamic data were acquired on a 3Tesla clinical MR system to monitor the spatial and temporal distributions of HP αKG and its metabolic product HP 2HG. RESULTS: Significantly higher levels of HP αKG were observed in tumors as compared to normal brain at all time points ($*p < 0.05$), suggesting higher delivery and retention of HP αKG in tumors. Furthermore, HP αKG levels were not significantly different between U87IDHwt and U87IDHmut tumors, indicating that substrate delivery is independent of tumor IDH1 status. Most importantly, HP 2HG originating from the conversion of HP αKG by mutant IDH1 was detected at 20s post HP αKG injection in all mutant tumors, but never in wild-type tumors, or in normal brain or blood ($*p < 0.05$). HP 2HG production therefore distinguished mutant IDH1 and wild-type tumors in vivo. In conclusion, $^{13}$C MRS of HP αKG informs on IDH1 mutational status and activity non-invasively in situ, and complements $^1$H MRS methods that detect steady-state 2HG levels. This method could help in diagnosis, prognosis and studies of response to mutant IDH1-targeted therapies.

### OM-014. GENOMIC ANALYSIS OF TRAF7-ONLY MUTANT MENINGIOMAS REVEALS NOVEL DRIVER MUTATIONS

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OBJECTIVE: Meningiomas are the most common primary brain tumors. We and others recently discovered that mutations in 5 genes (NF2, AKT1, TRAF7, KLF4, and SMO) and/or chromosome 22 loss explain nearly 80% of Grade I meningioma formation. Approximately one-fourth of meningiomas harbor TRAF7 mutations, which commonly co-occur with recurrent $AKT1^{E17K}$ or $KLF4^{K409Q}$ mutations but are mutually exclusive of NF2 mutations. In our original cohort of 300 meningiomas, 23.6% ($n = 17/72$) of TRAF7 mutant tumors did not harbor either $AKT1^{E17K}$ or $KLF4^{K409Q}$ co-mutations. We hypothesized that genomic analysis of TRAF7-only mutant meningiomas will enrich for the discovery of novel driver mutations, with implications for targeted pharmacotherapies. METHODS: We have performed comprehensive next-generation genomic analysis of meningioma samples including exome sequencing ($n = 71$), copy number (including chromosome 22), and gene expression analyses. RESULTS: Novel, recurrent intronic splicing mutations were discovered in 7.8% ($n = 6/77$) of TRAF7 mutant meningiomas. Consistent with our previous study, the majority of the remaining TRAF7 mutations were highly-recurrent missenses affecting the WD-40 repeat domains. Our analysis revealed new mutations to co-occur with TRAF7 mutations, including mutations known to activate PI3K signaling (ex. $PIK3R1^{del576\text{-}577}$, $PIK3CA^{108\text{-}111del}$, $PIK3CA^{E545K}$) and in CREBBP, whose loss has been reported in a variety of tumors.

CONCLUSION: Half of non-NF2 meningiomas harbor TRAF7 mutations, and characterizing this tumor subgroup has potentially important therapeutic implications. We have identified novel, recurrent splicing mutations in TRAF7, pointing to a tumor-suppressor role in meningioma formation. Additionally, we found new co-mutations that activate PI3K signaling, a pathway for which there are multiple pharmacological inhibitors in clinical trials.

### OM-015. ADULT GLIOMAS DISPLAY DIFFERENT DNA COPY NUMBER ALTERATIONS, PROGNOSTIC FACTORS, AND GENDER ASSOCIATION DEPENDING ON GRADE AND IDH STATUS

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BACKGROUND: IDH and grade define biologically and clinically distinct subgroups of gliomas. We describe differences in copy number alterations(CNA), gender distribution, and prognostic chromosomal changes in gliomas stratified by IDH status and grade. METHODS: CNA were detected by molecular inversion probe(Affymetrix) and analyzed with Nexus Copy Number Software(BioDiscovery). DNA was extracted from 94 patient FFPE samples including grade 2-3(17 $IDH1^{wt}$ and 28 $IDH1^{mut}$) and GBM(25 $IDH1^{wt}$ and 24 $IDH1^{mut}$). Multivariate stepwise Cox regression was used for survival analyses. RESULTS: Females were more likely to have grade 2-3 $IDH1^{mut}$ gliomas(47% vs 20%) and amplification of MET and PDGFRA and less likely to have CDKN2A deletion. When stratifying by grade and IDH status, the gender difference in CDKN2A loss was seen in all subgroups except for $IDH1^{mut}$ 2-3 gliomas, and only $IDH1^{mut}$ gliomas showed a gender difference in MET amplification. The gender difference in PDGFRA amplification disappeared after accounting for IDH status and grade. In grade 2-3 $IDH1^{mut}$ gliomas, NF1 LOH was more likely in males and there was a trend toward females having more EGFR amplification. In the overall survival analysis, IDH status and grade were the most significant factors, with CDKN2A loss and TP53 LOH being borderline significant. Multivariate analysis demonstrated that prognostic CNAs differed by subgroup. PTEN deletion and chromothripsis were significant prognostic factors in $IDH1^{wt}$ GBM, PTEN deletion and MDM2 amplification were significant prognostic factors in $IDH1^{wt}$ grade 2-3 gliomas, CDKN2A locus status and EGFR amplification were significant prognostic factors in $IDH1^{mut}$ grade 2-3 gliomas, and TP53 LOH and NF1 locus status were significant prognostic factors in $IDH1^{mut}$ GBM. CONCLUSIONS: While requiring external validation, these findings suggest both gender and CNA prognostic associations may differ significantly across glioma grade and molecular subtypes. These observations may help to identify clinically relevant therapeutic targets within these distinct subgroups.

### OM-016. PROGNOSTIC AND PREDICTIVE ROLE OF VEGF-A AND OF THE DIFFUSIBLE ISOFORM VEGF-121 IN PATIENTS WITH GLIOBLASTOMA

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BACKGROUND: VEGF-A is a key regulator of angiogenesis in glioblastoma (GBM). It is released by bulk tumor cells and by GBM stem-like cells (GSCs) in the "vascular niche". The importance of VEGF-A in GBM is underscored by the effectiveness of FDA-approved bevacizumab treatment. The VEGF-A gene can be alternatively spliced to produce isoforms with different biological properties. VEGF-121 is freely diffusible, being devoid of extracellular matrix (ECM)-binding domains. The prognostic role of VEGF-A expression in GBM is controversial; moreover, evidence is still lacking of the prognostic impact of VEGF-A isoforms and of their predictive role for anti-angiogenic therapy. METHODS: In a cohort of 16 primary GBM patients, we determined the expression of VEGF-A and VEGF-121 both on deparaffinized tumor samples and on tumor-derived GSCs using real-time RT-PCR. We also determined VEGF-A and VEGF-121 levels on an independent cohort of 17 patients treated with bevacizumab for recurrent GBM. RESULTS: A significant correlation was found for VEGF-121 levels (but not for VEGF-A) between tumors and GSCs. VEGF-121 levels in tumor, normalized per the amount of total VEGF-A, were significantly higher in patients surviving more than 1 year. A ratio VEGF-121/VEGF-A $> 2.5\%$ was a significantly good prognosticator ($p = 0.0025$) for survival. No correlations were found between tumor VEGF-A, GSC VEGF-A, GSC VEGF-121, and survival. Surprisingly, in the cohort of recurrent GBMs treated with bevacizumab VEGF-121 was inversely correlated with survival ($p = 0.01$), and patients displaying progression-free survival $> 12$ mos had significantly lower amounts of VEGF-121 ($p = 0.01$) and VEGF-A ($p = 0.04$). CONCLUSIONS: This study suggests that ECM-bound isoforms of VEGF, which have been associated with the generation of intratumoral microvessels, are the most clinically relevant target of anti-angiogenetic therapies. The role played by diffusible VEGF-121 is puzzling because it seems to hold a favorable value for survival but to predict poor response to bevacizumab therapy.

### OM-017. THE PROACTIVE-GLIOMA PROGRAM AT MD ANDERSON: GATEWAY TO PERSONALIZED MEDICINE TRIALS FOR GLIOMA

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To facilitate the rapid identification of patients with specific gene mutations and their enrollment into personalized medicine clinical trials for the treatment of glioma, we established the Prospective Assessment of Correlative and Tissue Biomarkers in Glioma Patients (The PROACTIVE-Glioma Program). Patients undergoing surgery for grade II, III or IV glioma were identified and consented. In collaboration with the Institute of Personalized Cancer Therapy (IPCT) at MD Anderson, DNA was extracted from archival FFPE tumor tissue and hotspot mutation testing was performed in the Clinical Laboratory Improvement Amendments (CLIA) environment on Sequenom or Ion Torrent platforms. This was followed by deep exome sequencing of approximately 200 cancer related genes in the research environment. In the first 8 months, over 250 grade II, III and IV glioma specimens were collected from patients who underwent surgery for newly diagnosed or recurrent glioma. Blood specimens were obtained for plasma and germline DNA analysis. Clinical demographic and treatment history are collected and entered into a custom database. Over 80 patients have at least two surgical specimens. The most common hot spot mutations/variants identified in the first 137 patients analyzed in the CLIA laboratory include IDH1 (28%), TP53 (33%), MET (8%), KDR (5%), EGFR (5%), and PIK3CA (4.4%). A subset of tumors has undergone more extensive testing in the research environment for 200 gene mutations and copy number variant analyses. Genomic testing in a CLIA environment is feasible on archival tumor tissue. Identification of patients with specific gene mutations at the time of diagnosis may facilitate their enrollment onto personalized clinical trials at the time of tumor recurrence.

### OM-018. A TELOMERASE-BASED ASSAY DETECTS CIRCULATING TUMOR CELLS IN THE PERIPHERAL BLOOD OF PRIMARY BRAIN TUMOR PATIENTS

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Circulating tumor cell (CTC) assays offer the potential to monitor disease status, gauge prognosis, and guide treatment decisions for patients with cancer with minimal invasiveness. CTC assays for patients with brain tumors (including high-grade glioma and Glioblastoma multiforme (GBM)) however have been complicated by the lack of surface expression of common biomarkers such as EpCAM to facilitate CTC separation and subsequent detection. We describe an alternative strategy utilizing an adenoviral detection system in which CTCs are identified based on the presence of telomerase activity, which successfully detects, for the first time, CTCs in patients with primary brain tumors. Pilot results suggest that serial evaluation of CTCs via this assay may potentially assist clinical interpretation of treatment response in patients undergoing radiation therapy, such as differentiating between pseudoprogression and true tumor progression.

### OM-019. OMICS ANALYSIS OF THE ANTI-GLIOMA EFFECT BY COMBINATION THERAPY OF VASCULOSTATIN EXPRESSING ONCOLYTIC VIRUS AND CILENGITIDE

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OBJECTIVE: The treatment of malignant gliomas has involved a combination of surgery, radiation, and chemotherapy, yet these modalities rarely extend the life of patients to more than one year from diagnosis. Oncolytic viral (OV) therapy has been considered as a promising treatment modality for malignant glioma. Previously, vasculostatin (the fragment of brain-specific angiogenesis inhibitor-1; BAI-1)-expressing oncolytic HSV-1 (RAMBO; Rapid Antiangiogenesis Mediated By Oncolytic virus) has showed potent antitumor effect against malignant glioma. Cilengitide (EMD121974), an inhibitor of integrins, has also demonstrated preclinical efficacy against malignant glioma. In this study, combination treatment of RAMBO and cilengitide shows stronger effect than each monotherapy, and we investigated the mechanism of anti-glioma effect of combination of RAMBO and cilengitide using DNA microarray. SUBJECTS AND METHODS: U87dEGFR (human malignant glioma cell line) cells were used for this experiment. The cells were treated by cilegnitide and combination of RAMBO and cilengitide in vitro, and they were harvested 16 hours for respective treatments, and mRNA has been extracted. Gene expression analysis, gene ontology analysis, and pathway analysis were performed with DNA microarray (CodeLink™ Human Whole Genome Bioarray). RESULTS: The expression of 981 genes was changed after combination treatment. The expression of 339 genes was up-regulated 4-fold more and the expression of 642 genes was down-regulated by 0.25-fold compared to the case of cilengitide monotherapy. In gene ontology analysis, genes which were associated with 'induction of apoptosis by intracellular signals' were over-represented. In pathway analysis, 'apoptosis' was over-represented. BCL2-like11 and DNA fragmentation factor genes were up-regulated. CONCLUSION: This study showed that omics analysis indicated a mechanism of anti-glioma effect of new vasculostatin-expressing OV therapy enhanced by cilengitide. Genes associated with apoptosis were over-represented by combination treatment of RAMBO with cilengitide.

### OM-020. POLY-OMIC PROFILING OF GLIOBLASTOMA LONG-TERM SURVIVORS REVEALS A NOVEL CHEMOTHERAPY-REFRACTORY, SLOW-CYCLING SUBGROUP

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Although treatment of glioblastoma is constantly improved by neurosurgical innovations and refined therapy regimens, it ultimately remains a fatal disease. Despite this dismal prognosis with a median survival of only 15 months, a subset of patients (3–5 %) survives for more than three years. Uncovering the molecular features which characterize this group of long-term survivors might identify novel prognostic biomarkers, point towards new therapeutic targets and provide further insights into the complex biology of glioblastoma. Employing different microarray platforms, we assessed DNA methylation as well as mRNA and miRNA expression on a genome-wide scale. Our study sample consisted of 10 long-term (LTS) and 7 short-term (STS) survivors and was highly selected in order to exclude IDH1 mutation as a prognostic factor. Furthermore, only patients who received concomitant radio- and chemotherapy as well as adjuvant chemotherapy were included. The top 44 differentially expressed genes served as a LTS signature which was subsequently used to validate our findings in a large dataset obtained from the TCGA ($n = 419$). Using k-means clustering, we identified two subgroups within the proneural subtype which significantly differed in survival, even when correcting for IDH1 mutation. The subgroups defined by the mRNA, miRNA and DNA methylation signatures also showed significant overlap, suggesting an underlying, biologically distinct phenotype. We also noted that patients in this group had an altered therapy response and did not benefit from chemotherapy. Further analysis revealed distinct transcriptional differences with a high number of genes which were differentially expressed between the subgroups. Metacore pathway analysis was used to assess the functional consequences of these transcriptional changes and suggested a less proliferative phenotype. Altogether, we were able to identify a new subgroup within proneural GBMs with a better prognosis, which is characterized by a less proliferative phenotype and also differs in therapy response.

### OM-021. CANCER STEM CELL TRANSCRIPTIONAL SUBTYPING OF GLIOBLASTOMA MULTIFORME CORRELATES WITH CLINICALLY RELEVANT MOLECULAR AND IMAGING PHENOTYPES

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Two distinct glioblastoma-derived cancer stem cells (CSC) have recently been described. CSC Type 1 (CSC1) is CD133 +, enriched with neurodevelopmental genes, grows as neurospheres in in vitro cultures, and displays a full stem-like phenotype. CSC Type 2 (CSC2) is CD133-, grows adherently in in vitro cultures, and demonstrates a more restricted stem-like phenotype. We compiled a total of 32 microarray gene expression profiles across 5 studies on GBM-derived CSC1 (18 profiles) and CSC2 (14 profiles) in order to determine gene expression signatures specific to each glioblastoma-derived cancer stem cell. We developed a Prediction Analysis of Microarrays (PAM) classifier to assign GBMs to one of the two CSC subtypes and, using data from The Cancer Genome Atlas (TCGA), assigned GBM CSC subtypes and correlated subtypes with molecular and imaging data. PAM identified 19 genes that are sufficient to classify GBM samples with high accuracy to one of the two CSC subtypes. We applied this predictor to a total of 426 GBM samples resulting in 300 (70%) versus 126 (30%) being classified as CSC1 vs. CSC2, respectively. CSC2 GBMs were enriched in mesenchymal GBMs ($P < 0.0001$). Virtually all CSC2 GBMs demonstrated a low proportion of non-enhancing tumor ($P < 0.0001$), were less infiltrative than CSC1 GBMs on the basis of enhancement/FLAIR ratio ($P = 0.004$), and were more likely to show a high proportion of necrosis ($P = 0.009$). Also, CSC1 GBMs were most likely to arise in the temporal lobe while CSC2 GBMs were most likely to arise in the frontal and parietal lobe. These data suggest that gene expression signatures derived from GBM CSCs may provide a novel means of subtyping GBM patients on the basis of potential cell-of-origin. Our results demonstrate clinically relevant phenotypic differences, both molecular and macroscopic, between GBMs from different presumed cancer stem cells of origin.

### OM-022. CREATING A RADIOGENOMICS MAP OF MULTI-OMICS AND QUANTITATIVE IMAGE FEATURES IN GLIOBLASTOMA MULTIFORME

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The goal of this work is to create mappings between quantitative image and genomic features for glioblastoma multiforme (GBM) and to assess the prognostic association of significant correlations. We obtained multi-omics data from 251 patients and MR image data from a subset of 55 patients in the Cancer Genome Atlas (TCGA) and The Cancer Imaging Archive (TCIA) GBM databases. A board certified neuroradiologist traced 2D regions of interest (ROI) around necrotic and enhanced parts of the largest lesion in a selected slice from T1 post-contrast MR, and around the region of hyperintensity obtained from the enhancement on the matched T2 FLAIR slice. These ROIs were used to compute quantitative image features from their shapes and pixel values. We used a module network algorithm that integrates copy number, DNA methylation and gene expression data into 100 co-expressed gene modules. We established a radiogenomics map by correlating these modules with the quantitative image features, and used significant module-image feature correlation for survival analysis using Cox proportional hazards modeling. A total of 28 quantitative image features were extracted for each of the necrosis, enhancement and edema ROIs in each patient. The radiogenomics map between modules and quantitative image features revealed 14, 10 and 16 significant gene-module associations with necrosis, enhancement and edema ROIs respectively. For example we found a significant correlation between Module 64, enriched with genes in neuronal differentiation, and the compactness of the necrosis ($p = 0.0145$). Also, we found that the amount of necrosis vs. enhancement or edema is correlated with Module 74, enriched in metabolism related genes ($p < 0.01$). Finally, we found e.g. that the compactness of the necrosis ROI is correlated with

poor survival ($p = 0.037$). Creating radiogenomics maps provides multi-scale insights by associating image features with molecular function. Moreover, these maps may provide additional insight for image features with prognostic correlations.

### OM-023. GLIOMETH: A NOVEL DNA METHYLATION SIGNATURE PREDICTS OVERALL SURVIVAL IN GLIOBLASTOMA MULTIFORME

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DNA methylation is a mechanism altering the normal state of cells implicated in many cancers. Currently the methylation status of MGMT is one of the most widely utilized clinical genetic tests performed on glioblastoma multiforme (GBM). While several global gene expression signatures have been developed, it is unclear if genome-wide DNA methylation signatures can predict prognosis in cancer. We used a computational algorithm (MethylMix) to analyze genome-wide DNA methylation in GBM data obtained from The Cancer Genome Atlas (TCGA). MethylMix identified a set of driver genes that met criteria for being both differential and functional. Differential refers to a difference in cancer methylation compared to normal tissue; functional refers to having a significant correlation with matched gene expression changes. We then used these MethylMix driver genes to build multivariate models of overall survival using linear regression and validated these models in independent data sets. Applying MethylMix and linear regression we identified a novel methylation signature predictive of overall survival, which we here define as the GLIOMETH signature. Interestingly, GLIOMETH did not include MGMT, suggesting that MGMT methylation is not essential to predict prognosis in GBM. GLIOMETH was prognostically significant even in a multivariate analysis with known prognostic covariates, including MGMT methylation. We validated GLIOMETH in two external DNA methylation data sets and two gene expression data sets, using a leveraging technique predicting methylation in terms of gene expression, showing also a significant survival correlation. Differential and functional DNA methylation is predictive of overall survival in GBM independent of known prognostic factors. We identified GLIOMETH as a DNA methylation signature that is predictive of overall survival in GBM, outperforming MGMT methylation. The GLIOMETH model validated across multiple independent DNA methylation and gene expression validation data sets demonstrating its robustness as an independent predictor of prognosis in GBM.

### OM-024. A RADIOGENOMIC ANALYSIS OF THE TCGA GLIOBLASTOMA DATA SET

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Genomic based approaches are increasingly being used to define subtypes of glioblastoma (GBM), in part due the availability of TCGA data. While TCGA is primarily focused on assessing molecular tumor signatures, pathology and radiology data is also available. In this talk, we will describe initial findings from a radio-genomic analysis of the TCGA GBMs. Although MRI imaging plays a key role in the diagnosis and in monitoring disease progression in GBM, it currently plays a limited role in patient stratification and treatment selection. Pre-operating imaging data from 89 patients was downloaded from The Cancer Imaging Archive, and tumor masks were generated on preoperative images. The volume of necrosis (N) and post-contrast-enhancement (CE) following gadolinium administration were assessed using a T1-weighted image and abnormal signal on a T2-FLAIR image was also quantified. These indices along with their derivatives (e.g. ratios such as N/CE) were then correlated against mRNA expression data obtained from the TCGA archive. Using this methodology, we generated a list of genes that were strongly correlated with a specific macroscopic property of the tumor; these gene lists then underwent enrichment analysis using Ingenuity Pathway Analysis. Preliminary analysis indicated that percent necrosis was strongly associated with over-expression of genes in the HIF-1 alpha pathway, anti-apoptosis, and mitochondrial dysfunction. Similar analysis is being formed for other imaging-based features and will be presented at the meeting. In conclusion, MRI data has the unique capability of describing the macroscopic environment of the tumor. While genomics provides an incredibly rich amount of data, tumor microenvironment also impacts gene expression. As the location from which a biopsy is often not known, as well as the considerable heterogeneity of GBMs, including macroscopic tumor properties may lead to better characterization of GBM tumor biology.

### OM-025. DECIPHERING A MOLECULAR SIGNATURE OF GLIOMA PROGRESSION USING MICRORNA EXPRESSION DATA

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Involvement of microRNAs in glioblastoma multiforme (GBM) has been well documented but less is known about microRNAs involved in lower grade glioma or in progression to higher grades of the disease. This study used Agilent 8x15K microRNA expression and Illumina microRNA sequencing data from The Cancer Genome Atlas to identify microRNAs associated with survival in both grade III (anaplastic astrocytoma) and grade IV (GBM) tumours. Differentially expressed microRNAs were compared between grades in order to identify potential markers of progression. Using a penalized regression model, nine microRNAs were identified as predictors of survival in GBM ($n = 482$). In the anaplastic astrocytoma group, mature microRNA sequencing data for two patient groups from the extremes of survival times were compared using a t-test. This analysis showed 35 microRNAs differentially regulated between the two cohorts. Five microRNAs; miR-34a, miR-148a, miR-222, miR-9 and miR-182, were associated with prognosis in both GBM and anaplastic astrocytoma. Furthermore, three of those microRNAs; miR-34a, miR-148a and miR-182, are encoded on regions of the genome previously implicated in glioma; proximal 1p LOH and chromosome 7 amplification respectively. Of the four microRNAs associated with survival in GBM but not astrocytoma; miR-145, miR-370, miR-10b and miR-124a, two showed altered expression patterns in GBM versus normal brain (NB) using a LIMMA test between 10 NB samples and 482 GBM samples. These microRNAs; miR-10b and miR-124a, have previously been reported to be involved in proliferation, migration and invasion in GBM. In conclusion, five microRNAs; miR-34a, miR-148a, miR-222, miR-9 and miR-182, have been identified to be associated with prognosis in both grade III and IV gliomas. These microRNAs may represent a 'signature' for prognosis prediction. MiR-10b and miR-124a are differentially expressed in GBM compared to normal brain and are implicated in prognosis in grade IV disease only.

### OM-026. THE GENETIC AND EPIGENETIC CONTEXT OF MGMT METHYLATION IN GLIOMA MAY IMPACT THE PREDICTIVE AND PROGNOSTIC VALUE

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BACKGROUND: The methylation status of the O6-methylguanine-DNA methyltransferase (MGMT) gene is an important predictive biomarker for benefit from alkylating agent therapy in glioblastoma (GBM). While MGMT methylation seems to have a strictly predictive effect in GBM, a strong prognostic effect has been observed in grade III glioma. Distinct epigenetic features and copy number variations (CNV) are associated with GBM and glioma III that are suspected to modulate the apparent MGMT associated treatment resistance. GBM exhibit frequent deletions of CHR 10, on which MGMT resides (10q26), while a characteristic feature of grade III glioma is the presence of a CpG island methylator phenotype (CIMP) that is associated with *MGMT* methylation. METHODS: DNA methylation data (Infinium human methylation BeadChip, HM-27K and 450K) were publically available for 800 GBM and grade II/III glioma and respective gene expression for 630 patient samples thereof. The MGMT methylation status was determined with the previously published MGMT-STP27 model (Bady et al. 2012). CIMP classification was obtained by clustering procedures of DNA methylation data, and CNV events were identified using a newly developed method based on the same platform. RESULTS: DNA methylation data was a good source for efficient prediction of CNV (e.g. EGFR amplification or 1p/19q codeletion). DNA methylation profiles segregated glioma grade II/ III into clear-cut genetically and pathologically distinct subgroups. The deletion of the MGMT chromosomal region significantly affected MGMT expression in grade III glioma even with MGMT methylation, while the latter was not observed in GBM. CIMP tumors, grade III or GBM, showed infrequent CHR 10 deletions. CONCLUSION: In grade III glioma CNV reducing the MGMT copy number further reduces MGMT expression in methylated tumors,

hence MGMT methylation in grade III gliomas without deletions on CHR 10 may not completely shut down MGMT expression and patients may benefit less from alkylating agent therapy.

### OM-027. A RETROSPECTIVE REVIEW OF THE INFLUENCE OF QUANTITATIVE MGMT METHYLATION ON SURVIVAL AFTER CHEMORADIOTHERAPY FOR PATIENTS WITH GLIOBLASTOMA

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INTRODUCTION: Bristol Genetics Laboratory has offered MGMT methylation analysis in glioma patients by MS-PCR (2005) and pyrosequencing (2011). Although documented in trials, the correlation between MGMT methylation and overall survival (OS) is less well reported in clinical practice. We investigated this correlation in our patient group and determined the level of methylation which was clinically significant. METHOD: DNA was extracted from formalin fixed paraffin embedded tumour tissue from 80 diagnostic samples from glioblastoma patients. Bisulphite modification and pyrosequencing assessed MGMT promoter % methylation at 12 CpG sites. Patients were treated with radiotherapy and temozolomide and overall survival (OS) recorded. RESULTS: Using our current strategy (<9% unmethylated (U), 10-20% weakly methylated (WM) and >21% methylated (M) based on validation of pyrosequencing against MS-PCR analysis), 32 patients were U (40%), 13 WM (16%) and 35 M (44%). Median overall survival (MOS) was significantly different; M (21 months), WM (16 months) and U (12.5 months) (Chi-square test, $p = 0.0003$). The WM group did not produce a clear survival curve. The best statistical fit was obtained with two data groupings at M >11% (Chi-square test, $p = <0.0001$) which gave a MOS of 20 months for M (58% patients) and 12 months for U. Age and gender did not significantly affect survival. CONCLUSION: Comparison of MGMT promoter methylation and overall survival showed a significant difference between M and U patients. A methylation cut off level of >11% provides best statistical fit and our routine testing strategy should be refined. The significance of % tumour content and methylation of individual CpG sites will be discussed.

### OM-028. EGFR AND EGFRvIII EXPRESSION IN GLIOBLASTOMA

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INTRODUCTION: Up to half of primary glioblastomas (GBM) show Epidermal Growth Factor Receptor (EGFR) (7p12) amplification, overexpression and/or mutations. EGFR activation promotes cellular proliferation via activation of MAPK and PI3K-Akt pathways. EGFR inhibitor therapy is under investigation for GBM. EGFRvIII is the most common variant resulting from an in-frame deletion of 801bp, leading to constitutively active EGFR. An EGFRvIII vaccine is currently in phase II/III trials. METHOD: 51 glioblastoma samples were retrospectively tested for EGFR amplification and EGFRvIII expression by immunohistochemistry (IHC), fluorescent in-situ hybridisation (FISH) and reverse-transcriptase PCR (RT-PCR), to determine the frequency of EGFR alterations and compare assays as potential tools for diagnostic use. RESULTS: 31% (16) samples were positive for EGFRvIII both by IHC and RT-PCR. Of these, 13 (81%) were also EGFR amplified and 3 (19%) were not EGFR amplified but had low-level EGFRvIII expression. 5 cases were initially negative on RT-PCR using whole sections but positive when repeated on macro-dissected material. Heterogeneity for EGFRvIII was observed by IHC. Interestingly, one patient had low level EGFRvIII expression which was later EGFRvIII negative at relapse. 16% (8) samples were EGFR amplified and EGFRvIII negative. 3 cases (6%) were discordant between methods; 2 between IHC and FISH for EGFR amplification and 1 positive for EGFRvIII by IHC only. CONCLUSION: In our cohort, 47% of GBM showed EGFR alterations, including 31% with EGFRvIII. There was high concordance between IHC, FISH and RT-PCR (94%) as diagnostic methods. These results are key for selecting patients for novel individualised anti-EGFR therapies.

### OM-029. MGMT PROMOTER METHYLATION IS PRESENT IN LOW GRADE GLIOMA (WHO GRADE II/III) AND GLIOBLASTOMA MULTIFORME IN THE ABSENCE OF IDH MUTATIONS

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INTRODUCTION: IDH mutation is an early event in gliomagenesis. Mutated IDH is believed to predispose glioma cells to extensive DNA hypermethylation because the abnormal IDH product 2-HG inhibits histone demethylation. Methylation of the MGMT gene promoter silences gene expression, increasing progression free and overall survival for patients treated with temozolamide (TMZ) chemotherapy. We compared the expression of MGMT promoter methylation and mutated IDH in a large cohort of glioma patients. METHOD: 88 samples from 71 patients were identified: 31 samples were insufficient for genetic analysis. For immunohistochemistry: sections were stained with anti-IDH1R132H antibody. For sequencing: DNA was extracted from fresh, frozen tissue. Sanger sequencing codon 132 IDH1 and codon 172 IDH2 was carried out. MGMT methylation status was assessed by methylation specific PCR. RESULTS: In our cohort, there is similar percentage of MGMT promoter methylation between WHO Grade II/III glioma and GBM (32/43 [74%] vs 16/28 [57%]), $p = .194$). In WHO Grade II/III gliomas alone, 20/21 (95%) of IDH mutation positive cases show MGMT promoter methylation compared with 6/11 (55%) of IDH mutation negative cases showing MGMT promoter methylation ($p = .011$). The presence of mutated IDH and MGMT promoter methylation together is associated with a significantly prolonged median survival in these tumours. For the GBM cases, 2/2 (100%) with IDH mutations show MGMT promoter methylation compared with 11/21 (52%) of IDH mutation negative cases ($p = .486$). CONCLUSION: Our results show a similar percentage of MGMT promoter methylation in WHO Grade II/III glioma compared with GBM. IDH mutations are overrepresented and significantly associated with MGMT promoter methylation in lower grade gliomas, but not in GBM. Our results suggest that alternate non-IDH mediated epigenetic mechanisms may be acting to silence MGMT expression in both low grade glioma and GBM. This has important implications for our understanding of glioma recurrence post TMZ therapy.

### OM-030. EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF CYSTEINE-RICH PROTEIN 61 IN GLIOBLASTOMA

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OBJECTIVE: Glioblastoma is the most common type of primary brain tumor in adults and remains extremely lethal. Recently, the research efforts in identifying prognostic molecular biomarkers for glioblastoma have increased. Cysteine-rich protein 61(CYR61) is one of the CCN family of matricellular proteins which promotes proliferation and angiogenesis in cancers through its interaction with several integrins. In this study, we investigated the relations of CYR61 and other prognostic factors in glioblastoma. METHODS: We analyzed tissues excised from 33 glioblastoma patients in adults treated at Okayama University between 2006 and 2011 (male, 17 female, 16; mean age, 59.2 years; range, 19-81 years). All patients underwent surgical resection followed by adjuvant radiation therapy and chemotherapy with temozolomide. Extent of resection was classified as gross total resection (GTR, ≥ 95% by volume), subtotal resection (STR, ≥80, < 95% by volume) or partial resection and biopsy (PR/ biopsy, < 80% by volume) based on magnetic resonance imaging performed less than 48 hours after surgery. Tumor samples were examined by immunohistochemistry for CYR61 expressions. Multivariate Cox proportional hazard model was used to identify possible prognostic factors. RESULTS: GTR, STR, and PR/ biopsy were achieved in 17 (51%), 5 (15%), and 11 (33%) cases, respectively. CYR61 expression was positive in 22 glioblastoma (66%). Median progression-free survival (PFS) and overall survival (OS) of patients with strong CYR61 expression was significantly shorter than those of patients with weak CYR61 expression (PFS: 6 months and 18 months, OS: 12 months and 27 months, respectively, $p < 0.005$). In a multivariate Cox analysis, CYR61 proved to be an independent prognostic factor of patient survival (PFS: HR = 2.77 (1.08 - 7.09), $p = 0.034$, OS: HR = 2.92 (1.00 - 8.49), $p = 0.049$). CONCLUSION: CYR61 might emerge as a strong candidate for a significant association with prognosis of glioblastoma.

### OM-031. IMAGE-GUIDED METABOLOMIC ANALYSIS OF 2-HYDROXYGLUTARATE IN IDH-MUTANT GLIOMAS

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GOAL: The goal of this study was to investigate the effect of 2HG on the cellular metabolome in patients diagnosed with a recurrent low-grade glioma. This was achieved by characterizing the differences in metabolic profiles between IDH mutant recurrent low-grade gliomas containing 2HG with wild-type IDH tumors using proton High- Resolution Magic Angle Spinning (1H HR-MAS) spectroscopy. METHODS: 110 patients received a pre-surgical research MR exam at the time of their recurrence that included diffusion, perfusion, and long-echo spectroscopy imaging. 242 image-guided tissue samples were obtained from areas of suspected tumor and bisected for metabolomic and histopathological analysis. Spectra were visually categorized as 2HG+ or 2HG-. RESULTS: A number of brain tumor metabolites were found to be significantly different in 2HG+ spectra, including total choline (tCho), N-acetyl-aspartate (NAA), glutathione (GSH), glycerophosphocholine (GPC), phosphocholine (PC), Myo-inositol (Myo-I), glutamine (Gln), phosphoethanolamine (PE), and glutamate (Glu). Differences in histological parameters between the two cohorts indicated increased mitosis (Ki-67), increased axonal disruption (SMI-31), and increased ratio of tumor cells / normal cells in 2HG+ tissue samples compared to 2HG-. These findings suggests that many of the metabolite differences in 2HG+ tumors may be due to increased cellularity in IDH mutant lesions. CONCLUSIONS: This research provides insight with regard to the differences in cellularity and metabolism in IDH mutant versus IDH wild type tumors. This information may be useful in developing surrogates for 2HG levels and designing imaging protocols for monitoring treatments for low-grade brain tumors, especially those that are being designed to target the IDH-pathway.

### OM-032. GLIOMA PATIENT TISSUE BIOMARKERS FOR HYPOXIA AND VASCULARITY TAKEN FROM SPECIFIC INTRATUMORAL REGIONS CORRELATE WITH PREOPERATIVE PERFUSION MRI AND ARE PREDICTIVE OF PATIENT OUTCOME

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INTRODUCTION: Dynamic contrast-enhanced MRI (DCE-MRI) data has the potential to noninvasively map parameters relevant to tumor hypoxia, blood flow, and vascularity. In this prospective pilot study, we used preoperative imaging to correlate specific glioma intratumoral regions with molecular markers of hypoxia, tumor vascularity, and tumor proliferation and predict patient outcome. METHODS: We generated maps of tumor blood flow (F), extraction fraction (E), permeability-surface area product (PS), transfer constant (Ktrans), washout rate (kep), interstitial volume (Ve), blood volume (Vb), capillary transit time (Tc), and capillary heterogeneity (α-1) from pre-operative DCE-MRI. Intraoperative navigation was used to obtain tissue samples from areas including peritumoral edema (PE), active tumor (AT), hypoxic penumbra (HP) and necrotic core (NC) which were evaluated for the expression of hypoxia inducible factor 1a (HIF-1a), vascular endothelial growth factor (VEGF), carbonic anhydrase nine (CA-IX), glucose transporter-1 (GLUT-1), vascularity, and tumor proliferation. RESULTS: Patient survival was found to correlate with DCE parameters α-1 in AT ($p = 0.005$), and Ve in areas of PE ($p = 0.020$). DCE parameters and tissue markers that were correlated included Vb and VEGF ($R^2 = 0.475$, $p = 0.0092$) and HIF-1 ($R^2 = 0.323$, $p = 0.043$) expression in AT, Tc and HIF-1 in AT ($R^2 = 0.527$, $p = 0.005$), HP ($R^2 = 0.761$, $p = 0.023$) and PE ($R^2 = 0.696$, $p = 0.0052$), and VEGF in AT ($R^2 = 0.346$, $p = 0.034$) and HP ($R^2 = 0.793$, $p = 0.0173$). α-1 is correlated with VEGF in AT ($R^2 = 0.442$, $p = 0.013$) and PE ($R^2 = 0.951$, $p < 0.0001$) and Kep is correlated with VEGF in PE ($R^2 = 0.579$, $p = 0.0065$) and NC ($R^2 = 0.868$, $p = 0.0069$). In addition, MIB-1 index is correlated with VEGF expression in HP ($R^2 = 0.7933$, $p = 0.0071$) and PE ($R^2 = 0.4546$, $p = 0.033$). CONCLUSIONS: Our pilot study findings suggest that with further work it may be possible to use DCE-MRI to make noninvasive preoperative predictions of areas of tumor with increased hypoxia and proliferation. This has the potential to both make unprecedented prognostic decisions and to guide therapies to specific tumor areas.

### OM-033. THERAPY-INDUCED EVOLUTION OF LOW-GRADE GLIOMA GENOMES DURING MALIGNANT PROGRESSION

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Low-grade gliomas are slow-growing tumors that often undergo malignant progression to an aggressive high-grade glioblastoma (GBM) with a significantly worse prognosis. Treatment options after surgical resection include temozolomide (TMZ), an alkylating chemotherapeutic which is cytotoxic but can induce C > T/G > A transition mutations when DNA mismatch repair is deficient. However, the extent and clinical impact of TMZ-associated mutagenesis is poorly understood. To investigate the genomic evolution of recurrent tumors and the contribution of TMZ-induced mutagenesis to their mutational landscape, we sequenced the exomes of primary low-grade gliomas and their recurrences resected up to 11 years later. We found that many of the tumors from patients treated after surgery with TMZ became hypermutated and subsequently recurred as GBM. Greater than 95% of these mutations were C > T/G > A transitions that predominantly occurred at CpC and CpT dinucleotides, a pattern characteristic of TMZ-induced mutagenesis. In these hypermutated recurrent GBMs, TMZ-associated mutations altered the function of key cancer genes in pathways involved in malignant progression. We identified activating mutations in oncogenes like *MTOR* and *PIK3CA,* as well as inactivating mutations in tumor suppressors such as *CDKN2A*, *PTEN*, and *RB1*, all bearing the signature TMZ-induced mutagenesis. Thus, the exposure of grade II astrocytic gliomas to adjuvant TMZ is associated with the acquisition of a massive mutational burden that alters the function of key cancer genes and their cognate pathways, potentially driving malignant progression. These findings suggest that this widely used therapeutic agent has the potential to accelerate tumor evolution with unintended clinical and biological consequences.

### OM-034. CLONAL EVOLUTION IN A HIGHLY RECURRENT ASTROBLASTOMA OF CHILDHOOD

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Astroblastoma is a rare glial tumour of unknown origin, with no established WHO grade, and controversial claims to being a distinct entity. These lesions are characterised by the presence of the astroblastic pseudorosettes composed of tumour cells with a prominent process extending to a central blood vessel and perivascular hyalinization. These lesions most commonly present in children and young adults, and may have a better clinical outcome than glioblastoma. We have studied a unique case of astroblastoma arising in a 6 year old girl, with multiple recurrences over a period of 10 years, by whole exome sequencing and 450k Illumina methylation profiling. Initial presentation of a fronto-parietal tumour was followed after multiple rounds of surgery and chemoradiotherapy with recurrences in the left frontal, left parietal parafalcine, left temporal, sphenoid and nasal locations. The patient died age 16. The tumours studied were not mutated for *H3F3A*/*HIST1H3B*, *ATRX*/*DAXX*, *IDH1/2* or *TP53*. Copy number profiles showed gains of 9q,15q, and losses of 9q,10,13q and 14q, with a high degree of divergence over each subsequent recurrence. A total of 331 somatic variants were observed in any tumour sample. Of these, a core list of 29 genes were somatically mutated in all recurrences, indicating a common origin for all tumour samples. These included the MAP-kinase signalling pathway member *MAP4K5*, and *XRRA1*, a protein involved in radiation resistance. Early changes which were selected against over time included mutations in *ARID1B,* involved in chromatin remodeling, and *REC8*, essential for sister chromatid adhesion. Late acquired events included mutations in *DDX11*, an RNA helicase associated Warsaw breakage syndrome. These data present a remarkably diverse clonal evolution of astroblastoma during childhood, and argue for its consideration as a distinct biological entity to paediatric glioblastoma, with its driving molecular alterations targeting chromosomal integrity.

## OM-035. THE MEDULLOBLASTOMA METHYLOME REVEALS NEW EPIGENETIC REGULATORY MECHANISMS

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Much has recently been discovered with respect to genomic and transcriptomic alterations underlying medulloblastoma, the most common embryonal brain tumor. One of the most important insights is that medulloblastoma is not a single disease, but rather comprises four core molecular subgroups. We therefore sought to characterize global epigenetic alterations occurring in these medulloblastoma subgroups as part of the International Cancer Genome Project (ICGC) PedBrain Tumor project. In order to get a global, base-resolution profile of the medulloblastoma methylome, we performed high-coverage whole-genome bisulfite sequencing on 34 primary tumors and 8 normal cerebellum samples. To supplement this, we conducted genome-wide methylome analysis on >300 primary medulloblastomas (frozen and FFPE) using the Illumina Infinium HumanMethylation450 bead array. Matched transcriptome data from either microarrays or RNA sequencing, as well as miRNA sequencing data, was available for over 100 tumors, allowing us to correlate methylation with gene expression. DNA methylation was found to be correlated with overall expression levels and alternative isoforms in numerous regions. Interestingly, the strongest association was not at classical CpG islands, but downstream of transcription start sites. Differential methylation/expression between subgroups was observed for many known subgroup markers, but also novel candidiate genes (e.g. ARID1B, LIN28B) and miRNAs. Large-scale partially methylated domains (PMDs), identified in WNT and Group 3 tumors, were correlated with low gene expression and an increased somatic mutation rate. This study provides an extremely detailed view of the methylomic landscape of medulloblastoma, revealing novel insights into the epigenetic regulation of subgroup-specific mRNA and miRNA expression. Furthermore, the scope of the study has allowed us to identify associations between DNA methylation, gene expression, and alternative splicing / promoter usage which have wider implications for basic biology. These data will provide a basis both for future research studies and for the development of enhanced therapeutic modalities.

## OM-036. RECEPTOR/RAS/PI3K/Akt/mTOR VARIATIONS IN Akt PATHWAY SUBTYPES SUGGEST THERAPEUTIC ALTERNATIVES

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Several receptor/PI3K/Akt/mTOR pathway inhibitors performed well in Glioblastoma (GBM) preclinical studies, but there were only sporadic responses in clinical trials. We hypothesized that variations in this pathway among GBM patients might underlie this result. We classified GBM based on Akt pathway genes in a test set of tumors, and 5 Akt pathway subtypes resulted. The subtypes fit previous classifications, but further divided classes, yielding new subtypes with differing clinical and molecular features. We expanded this work into data sets within The Cancer Genome Atlas (TCGA), and investigated variations in receptor/RAS/PI3K/Akt/mTOR activity among our subtypes. Here we concentrate on our Akt subgroup 4, which corresponds to the TCGA "classical" subgroup. TCGA reverse phase protein array data suggest p-EGFR and pERBB2 are dominant receptors signaling to SRC and STAT3 but not Akt and MAPK in Akt subgroup 4. The data suggest that combinations of EGFR/ERBB2, SRC and STAT3 inhibitors will be effective in this group of patients. Taken together, the results indicate significant variations in the receptor/RAS/PI3K/Akt/mTOR pathway between subtypes and suggest Akt classification will help select effective therapeutics for GBM patients.

## OM-037. IDENTIFICATION OF A GENE SIGNATURE DISTINCTIVE OF PRIMARY GLIOBLASTOMAS WITH SPATIAL RELATIONSHIP TO THE SUBVENTRICULAR ZONE

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OBJECTIVE: We have recently reported that spatial relationship of primary glioblastomas (pGBMs) to the subventricular zone (SVZ), the predominant neurogenic region of the adult brain, is associated with inferior patient survival and significantly increased protein expression of neural stem cell markers, suggesting localization-dependent differences in the clinical behavior and stem cell phenotype. We therefore hypothesized that there are distinctive localization-dependent gene expression patterns shedding further light into the molecular heterogeneity of pGBM. METHODS: RNA was extracted from histologically confirmed vital tumor samples of 36 pGBMs differing in their spatial relationship to the SVZ (contact: n = 17; no contact: n = 19) and utilized for mRNA microarray analysis (Illumina© HumanHT-12 v4 Expression Bead Chip). Data processing and statistical analysis was carried out employing R statistical computing software. Pathway analysis was conducted using MetaCore™ software. RESULTS: mRNA microarray analysis was able to identify a gene signature that distinguishes pGBMS contacting the SVZ from pGBMs without contact to the SVZ. Metacore™ pathway analysis of the top 288 differentially expressed genes revealed pathways associated with immune responses, Notch signaling, angiogenesis and the actin cytoskeleton to be the most differentially expressed pathways in pGBMs with respect to their spatial relationship to the SVZ. Apart from a significant overexpression of CD133 in pGBM contacting the SVZ, no clear-cut differential regulation of typical (tumor) stem cell markers or lineage markers was found. Localization-dependent gene expression patterns were distinct from the TCGA's molecular subtypes. CONCLUSION: In this study, gene expression patterns of pGBMs with or without contact to the SVZ were found to differ significantly in major pathways associated with gliomagenesis and immune responses, suggesting an outstanding role of tumor localization with presumably different cells of origin that contribute to the molecular heterogeneity of pGBMs. Further analysis is under way to validate and functionally characterize the most promising candidate genes.

## OM-038. IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH MALIGNANT TRANSFORMATION OF IDH-MUTATED LOW-GRADE ASTROCYTOMAS USING GENOME-WIDE COPY NUMBER VARIATION ANALYSIS

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OBJECTIVE: To identify copy number variations (CNVs) between IDH-mutated 1p/19q non-codeleted low-grade astrocytomas (LGA) and their consecutive chemo and radiation naïve secondary glioblastomas (sGBM). METHODS: LGA tissue, the consecutive sGBM, and matched blood samples were collected from seven glioma patients. Tissues were assessed for IDH1/2 mutations, MGMT promoter methylation, and 1p/19q-codeletion. Affymetrix Genome-Wide Human SNP Array 6.0 and Nexus (Biodiscovery) using SnpRank segmentation algorithm to identify CNVs were used. Twenty-five probes were required for a CNV to be called. For each segment, a minimum probe median of +0.2 (−0.2) was required to call a duplication (deletion). Genomic coordinates correspond to human genome build GRCh37 (ucsc hg19) and dbSNP build 131. RESULTS: Median follow up was 11.4years, median time to malignant progression 2.3years (1.5-9.9years) and median OS was 12.7years (10.1-15.3years). A total of 258 CNVs were identified comparing LGA vs. gDNA, 318 comparing sGBM vs. gDNA, and 427 comparing sGBM vs. LGA, including 460 amplifications, 510 deletions, and 33 LOH events. The most common CNV (4/7patients) comparing sGBM vs. LGA was chr13:49521235-70694175, which was a deletion that was also seen in the same patients when using gDNA as reference. In one of the four patients the deletion was already present in the LGA. Using Ensembl biomart, 188 genes fall into this region. Other CNVs found in at least three individuals were chr18:61130792-61135806 (amplification), chr3:84631171-84669615

(deletion) and several separate CNVs in the region of chr9:0-11398649 (deletions). DISCUSSION: We identified several regions putatively associated with IDH-mutated 1p/19q non-codeleted LGA progression to sGBM. Several genes were found in those regions, which have been previously reported to be associated with malignant transformation such as DOCK8, PTPRD, DMRT1 and CDH7. Importantly, several CNVs suggesting novel candidate genes involved in malignant transformation such as PCDH9 and 17, and RCBTB1 were identified in this analysis.

## OM-039. IDH1 STATUS AS A DIAGNOSTIC AND PROGNOSTIC MARKER FOR MALIGNANT ASTROCYTOMAS WITH LIMITED HISTOLOGICAL SPECIMEN: THE ROLE OF SURGICAL RESECTION, PATHOLOGICAL ANALYSIS AND MOLECULAR TESTING

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The accurate grading of malignant astrocytomas has significant prognostic and therapeutic implications. Traditional histopathological grading is limited by its subjective nature, regional tumor heterogeneity, and small tumor samples available for complete analysis. Here, we hypothesized that a critical tumor resection volume is needed for correct grading of astrocytomas by pathology, and for insufficient tissue sampling, IDH1 molecular testing can act as a complementary marker to improve diagnostic accuracy. Volumetric analysis were obtained using preoperative and postoperative MRI images and histological tissue samples from 402 patients with malignant astrocytomas who underwent craniotomies. IDH1 status was assessed by immunohistochemistry and sequencing. Histologically-confirmed GBM and AA were found in 246 and 156 patients, respectively. 121 patients had IDH1 mutations while 254 patients were IDH wildtypes. 27 patients with indeterminate IDH1 status were excluded. Patients with $>20$ cubic centimeters (cc) of the total tumor volume resected on MRI were found to have higher rate of GBM diagnosis as compared to $<20$cc (OR 2.57, 95% CI 1.6-4.06, $P < 0.0001$). In contrast, the rate of IDH1 status remained constant regardless of the volume resected (OR 0.81, 95% CI 0.48-1.36, $P<0.43$). The rate of GBM diagnosis is also 2-fold greater for individual surgical tumor specimen $>10$cc (OR 2.48, 95% CI 1.88-3.28, $P<0.0001$). In addition, the overall survival for AA patients with $>20$cc tumor resection on MRI is significantly better than those with $<20$cc tumor resected ($P < 0.05$), while no differences were observed in patients with GBM ($P < 0.4$), IDH1 wildtype ($P < 0.1$) or IDH1 mutants ($P < 0.88$). IDH1 status should be used for total resection volumes of $<20$cc to complement histopathologic diagnosis of malignant astrocytomas. Surgical specimen less than this minimal volume may result in the under-diagnosis of GBM simply based on histopathology analysis alone, with significant prognostication and therapeutic implications.

## OM-040. EXOME SEQUENCING OF CNS GERM CELL TUMORS REVEALS FREQUENT MUTATIONS IN KIT AND KRAS PATHWAYS AND JMJD GENES

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BACKGROUND/AIMS: CNS germ cell tumors are a group of rare heterogeneous brain tumors affecting mainly male adolescents and young adults with the highest incidence in Japan and other East Asian countries. Although the survival rate of pure germinoma is excellent, the outcome of nongerminomatous germ cell tumors (NGGCTs) is poor despite the use of multi-modality therapy. Thus there is an urgent need to develop novel therapeutics. METHODS: Initially we analyzed 28 cases of CNS germ cell tumors using whole-exome sequencing. A total of 180 somatic non-synonymous mutations and a subset of germline variants were validated using the Ion Torrent PGM. These mutations were subsequently validated in an independent set of 43 cases using the AmpliSeq platform. RESULTS: Somatic mutations of KIT were detected in 29% of cases, confirming previous studies. In an additional 38% of the cases, somatic mutations were detected in important downstream mediators of KIT signal transduction including KRAS, NRAS, MTOR, AKT3 and regulators, PTEN and NF1. Mutations in KIT were mutually exclusive with mutations in RAS. Additionally, 21% of the patients showed inactivation of tumor suppressor genes including TP53, BCORL1, PTEN, PTCH1. For the first time we report a significant enrichment of novel germline variants in the jumonji domain-containing (JMJD) genes found only in NGGCTs. Inactivation of TSGs was also preferentially present in NGGCTs. 91% of the pure germinomas showed pan-chromosomal uniparental disomy and chromosomal imbalance whereas 40% of the NGGCTs were chromosomally stable. CONCLUSION: These results have important implications for our understanding of the genetic complexity and heterogeneity underlying CNS germ cell tumors, and provide potential targets for the development of novel therapeutics. In addition, they also provide valuable insights into potential genetic predisposition to these tumors that could explain the ethnic differences in the incidence.

## OM-041. GENOME-WIDE CNV ANALYSIS IDENTIFIES LOCI PUTATIVELY ASSOCIATED WITH DELAYED TIME TO FIRST RECURRENCE IN IDH-MUTANT LOW-GRADE ASTROCYTOMAS

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OBJECTIVE: To identify copy number variations (CNVs) in IDH-mutated low-grade astrocytomas (LGA) predicting time to recurrence. METHODS: LGA tissues and germline DNA from 17 LGA patients were analyzed. Tumor samples were assessed for IDH1/2 mutations and 1p/19q-codeletion. Analysis was performed using Affymetrix Genome-Wide Human SNP Array 6.0 and aroma.affymetrix package for R. After preprocessing with CRMAv2 segmentation by circular binary segmentation, only segments with 10 or more probes were included in CNV analysis. CNVs were called if the probe mean in each segment was greater than 1.5 for amplifications, and less than 0.75 for deletions, using germline DNA as reference. Union of segment borders resulted in 638 total segments. A proportional hazards model was fit with months to first recurrence as dependent variable for segments identified in two or more individuals. Genomic positions correspond to human genome build GRCh37 (UCSC hg19) and dbSNP build 131. RESULTS: Two cases had a 1p/19q-codeletion. Segmental deletions on 1p and 19q were associated with time to recurrence (1p35.1-p36.1 and 19q13.2-q13.43). For the following analysis excluding 1p/19q codeleted cases median time to first recurrence ($n = 15$) was 23 months. 21 autosomal segments were found in two or more patients. The maximum number of individuals with an aberration for any segment was three (chr5:57329584-57333534, chr6:103737977-103762062, chr8:144309756-144310618). Two segments were suggestively associated with time to first recurrence: chr5:197445-57839813 (raw-$p = 0.085$; risk ratio $= 5.8$) and chr8:144309756-144310618 (raw-$p = 0.057$; risk ratio $= 5.3$). Both amplifications and deletions were called on those chromosome 5 and chromosome 8 segments. CONCLUSIONS: Suggestive associations of CNVs with time to first recurrence of IDH-mutated low-grade astrocytomas were identified. Segmental deletions on chromosomes 1 and 19 (when including 1p/19q codeleted cases) and aberrations on chromosomes 5 and 8 (in 1p/19q non-codeleted LGA) were suggestively associated with time to first recurrence.

## OM-042. PROGNOSTIC VALUES OF LONG NON-CODING RNA SIGNATURES IN GLIOBLASTOMA MULTIFORME

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INTRODUCTION: Long non-coding RNAs (lncRNAs) are mRNA-like transcripts that lack significant protein-coding abilities. Like microRNAs, lncRNA have been implicated in cancer biogenesis. We have previously shown that lncRNA expression profiles correlated with histological subtypes

and malignancy grades in human glioma.[1] The aim of this study is to further identify lncRNA signatures that have prognostic values in glioblastoma multiforme (GBM). MATERIALS AND METHODS: Using a lncRNA-mining approach,[1] we performed lncRNA expression profiling in 213 GBM specimens from The Cancer Genome Atlas (TCGA). These were randomly divided into a training (n = 107) and a testing set (n = 106). We analyzed the associations between lncRNA signatures and clinical outcomes in the training set, and validated the findings in the testing set. We also validated the identified lncRNA signature in another two independent GBM data sets from the Gene Expression Omnibus (GEO), which contained specimens from 68 and 101 patients, respectively. RESULTS: We identified a set of six lncRNAs that was significantly associated with overall survival in the training set. These included KIAA0495, PART1, MGC21881, MIAT, GAS5 and PAR5. Based on this six-lncRNA signature, the training-set patients could be classified into high-risk and low-risk subgroups with significantly different survival (HR =2.13, 95% CI = 1.38-3.29; P = 0.001). The prognostic value of this signature was confirmed in the testing set and the two independent data sets. Further analysis revealed that the prognostic value of this signature was independent of age and O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status. CONCLUSION: LncRNAs have potential roles in GBM pathogenesis and translational studies. Further investigations may focus on their functional roles and potential use in molecular subclassification of GBM. REFERENCES: 1. Zhang, X., et al. Long non-coding RNA expression profiles predict clinical phenotypes in glioma. Neurobiol Dis 2012; 48: 1-8.

### OM-043. PREDICTORS OF PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL IN IDH1-MUTANT GLIOMA PATIENTS

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IDH-mutated gliomas represent a distinct subtype among cerebral neoplasms, with improved prognosis compared to histologically-matched IDH-wild-type gliomas. Whether this improved prognosis is a result of intrinsic natural history, or response to treatment, is unknown. We retrospectively examined a cohort of IDH1-mutant glioma patients to determine predictors of overall survival (OS) and progression-free survival (PFS, measured from diagnosis to initiation of salvage therapy or death). Of 146 patients (60 WHO grade II, 65 grade III, 21 grade IV), 26 deaths were recorded in the cohort, with median follow-up in survivors of 4.6 years. Median PFS was 7.4 years (95% CI 6.0-8.9) with median follow-up in non-progressors of 4.1 years. We analyzed baseline clinical factors - including age, histological grade, extent of initial surgery (absence of residual enhancing disease vs. subtotal resection or biopsy), and receipt of initial adjuvant radiation therapy - for association with OS and PFS. Interestingly, we did not observe an association between initial histopathological grade and PFS (p = 0.428) or OS (p = 0.268), supporting the hypothesis that IDH-mutant gliomas represent a distinct and slowly-progressing neoplastic process compared to IDH-wild-type tumors. Both extent of surgical resection and initial adjuvant radiation therapy were significantly associated with improved OS (HR = 0.13 for surgery, HR = 0.31 for radiation) in a multivariate Cox model including age and histologic grade. We further identified a positive association between extent of surgical resection and PFS (median 7.9 years vs. 5.4 years, p = 0.033), which remained significant (HR = 0.57, p = 0.049) in a multivariate model. Initial extensive surgical resection and/or radiation therapy are associated with improved outcome in patients with IDH mutant gliomas. Our retrospective analyses have limitations due to the small number of grade IV tumors and need for longer follow-up. Nevertheless, this evidence mirrors prior studies in histologically-defined low-grade gliomas, suggesting a potential extension of this treatment paradigm to all IDH-mutant gliomas.

### OM-044. WHOLE EXOME SEQUENCING OF PAIRED GBM SPECIMENS FROM PATIENTS TREATED WITH CHEMORADIOTHERAPY

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Despite undeniable progress in the care and treatment of primary brain tumours, for most patients diagnosed with glioblastoma (GBM) prognosis is poor and less than 5% of newly diagnosed GBM survive more than 5 years. Standard treatment for GBM consists of combining the alkylating agent, temozolomide with radiotherapy (chemoradiotherapy). Methylation of the MGMT promoter region of the tumour is a strong predictor of benefit with chemoradiotherapy for a subgroup of patients, however the tumour eventually relapses. Understanding the resistance to chemoradiotherapy could facilitate the development of therapies that specifically target such resistance mechanisms. To understand treatment resistance acquired after chemoradiotherapy treatment and to identify genetic variations associated with glioblastoma relapse, we performed whole exome sequencing. DNA was extracted from frozen GBM tissue where the pre-treatment and the post-treatment tissue were available to allow comparison. Whole exome sequencing specifically targets the protein-coding region of the genome. This is the most functionally relevant genome region and most disease related variants are found residing within this region. We obtained >98% coverage of target region and an average sequencing depth of 102 for each sample. Among an average of 400 mutation events observed in post-treatment tumour for each patient, only a few mutations were found to be specific to temozolomide treatment. An independent cohort of paired patient specimens are being used for validation of the chemoradiotherapy-related changes identified in the relapsed tumour. Moreover, this study is designed to identify and validate new targets that contribute to the acquired resistance of temozolomide treatment. The results will lead to new therapies to be used as an adjunct to chemoradiotherapy and/or new therapies to treat the tumours after relapse.

### OM-045. DEVELOPMENT OF RECEPTOR TARGETED QUANTUM DOTS AS IN VITRO AND IN VIVO DIAGNOSTIC AGENT FOR GBM

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Glioblastoma Multiforme (GBM) tumors are known to express IL13Rα2, a receptor for interleukin-13, which is not expressed in normal tissues. Our earlier studies indicated the tumor specific binding and internalization property of the IL13 conjugated liposomes on the GBM tumors. In the present investigation quantum dots were utilized for the tumor and tumor associated receptor detection. Quantum dots are semiconductor nanomaterials in the size range of 5-20 nm. The distinct properties of the quantum dots like size tunable narrow emission spectra with wide excitation range, resistance to autobleaching and enhanced fluorescence half-lives makes the material suitable for non-invasive in *vivo* fluorescence measurement. In our present investigation IL13 protein was conjugated with carboxylated CdSe quantum dots bearing PEG groups on the surface. The material was characterized for the surface morphology, protein conjugation and particle size by Atomic Force Microscopy (AFM), agarose gel electrophoresis and particle size analyzer respectively. The affinity of the quantum dots towards the U251 glioma cells was also confirmed using the AFM method. Selective binding of the IL13 conjugated quantum dots to the glioma cells and glioma stem cells was evident when cultured as monolayer and this binding was inhibited when the receptors were blocked with excess IL13 protein. Cell proliferations assays were performed with the IL13 conjugated and non-targeted quantum dots . In vitro investigations were carried out to determine the aggregation phenomenon of these quantum dots in the presence of soluble receptor protein. When the IL13 conjugated quantum dots were exposed to soluble IL13Rα2 protein in nanomolar concentrations there was a tendency for the quantum dots to aggregate as evidenced by flow cytometry. This property can be utilized in the detection of soluble cancer associated IL13Ra2 receptor in the biological fluid. In vivo tumor diagnosis in an intracranial glioma tumor mouse model is in progress.

### OM-046. SINGLE CELL SEQUENCING RESOLVES CLONAL AND SUBCLONAL ALTERATIONS IN GLIOBLASTOMA

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Glioblastomas have been shown by histomorphologic, cytogenetic, and genomic studies to contain heterogeneous tumor cell populations. Such heterogeneity has been hypothesized to account for failure of targeted therapy in glioblastoma. In particular the mechanisms of resistance to EGFR inhibitors, the most common mutation target in this disease, remain unresolved. Here, we present a cell population-based method to characterize tumor heterogeneity by single-nucleus sequencing and allelotype-based clustering of subclonal

populations. Analysis of two primary glioblastomas reveals complex genomic architectures and hierarchical events leading to clonal and subclonal populations. The method allows all categories of genomic variation to be analyzed including somatic mutations, copy number, and rearrangements. Deletions in canonical GBM tumor suppressor genes were found to result from several mechanisms including simple bi-allelic deletion (PTEN), compound bi-allelic deletions, and complex multiple chromothripsis rearrangement events (CDKN2A). Moreover, our analysis reveals distinct patterns of somatic copy number alterations within glioblastoma subclones, and clonal EGFR amplification followed by independent subclone-specific evolution of distinct EGFR variant II (EGFRvIII) rearrangements. This heterogeneous evolution of a single gene has potential functional implications as we show the EGFR variant II protein exhibits transforming and inhibitor-sensitive properties similar to EGFRvIII. We conclude that multiple new categories of genomic heterogeneity are revealed by single cell sequencing, and provide evidence suggesting that EGFR alterations develop sequentially during tumor evolution and have variations that may contribute to resistance to current treatments. Single-nucleus sequencing can elucidate multiple forms of genomic heterogeneity in cancer and can resolve the complex heterogeneity in driver events obscured by conventional bulk analysis.

## OM-047. LONGITUDINAL EPIGENOMICS OF LOW-GRADE GLIOMAS DURING MALIGNANT PROGRESSION

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Low-grade gliomas (LGGs, WHO grade II) invariably recur following initial resection, frequently undergoing malignant progression to high-grade glioblastoma (GBM, WHO grade IV). Little is known about epigenomic changes over time in individual patients and their dependence on genetic changes associated with recurrence and malignant progression to GBM. To characterize the evolution of the DNA methylome within the context of genetically defined clonal evolution, we used whole genome bisulfite sequencing, Illumina HumanMethylation450 arrays and exome sequencing to profile primary LGGs and patient-matched recurrent tumors collected up to 11 years later. While IDH1-mutant tumors shared the glioma CpG island methylator phenotype (G-CIMP), diverse patterns of methylome evolution also emerged along with a common pattern of hypomethylation specifically associated with malignant progression to GBM. Integration of methylation profiles with temporal gene expression changes from RNA-seq identified increased expression of cell cycle genes as a common target of functional hypomethylation. This was consistent with a substantial increase in the number of actively cycling cells between primary and patient-matched recurrent tumors. The individual-specific evolution of the epigenome showed a remarkable concordance with the clonal architecture and genetic evolution defined by the exome sequencing in both temporally and spatially distinct samples, suggesting the two processes are intimately linked. We conclude that the epigenetic landscape is continually evolving coordinately with genetic evolution. These results offer new insights into temporal ordering of epigenetic changes in relation to acquisition of mutations, and suggest a new epigenetic mechanism that contributes to cell cycle dysregulation during malignant progression.

## OM-048. A FIVE GENE SIGNATURE TO PREDICT OUTCOME OF TRC-102 AND TEMOZOLOMIDE TREATMENT IN GLIOBLASTOMA

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Malignant glioma is highly aggressive, poorly chemoresponsive, and represents a management challenge. Temozolomide in combination with radiation is the standard-of-care treatment for glioma patients. Acquired or de novo drug resistance accounts for tumor relapse. Enhanced DNA repair capability is one of the main reasons why glioma cells are resistant to DNA damaging agents like temozolomide. Base Excision Repair (BER) is the major DNA repair pathway that modifies base lesions which arise due to alkylation, oxidation, deamination and depurination/depyrimidination of bases. TRC-102 or Methoxyamine is a potent inhibitor of BER pathway. It binds abasic sites and disrupts BER pathway. Combination treatment of TRC-102 and temozolomide is already in clinical trials for other solid tumors and seems promising for glioma therapy. There is an important caveat though for this treatment to work. BER-inhibitors will potentiate efficacy of radiation and/or alkylating agent chemotherapies optimally, and possibly only, if the tumor cells express high levels of monofunctional glycosylases to generate abasic sites and low levels of endonuclease which repair and remove abasic sites. Analysis gene expression profiles of key BER pathway genes in 428 samples from TCGA portrays 3.9% of patients harboring this "theoretical optimal" expression pattern indicative of BER-inhibitor vulnerability. Including three other important predictors of vulnerability to BER pathway inhibitors (TopoIIa, Smug1 and Neil3) further decreases this to 2.1%. These percentages are a conservative estimate and might be higher depending upon expression differences of other glycosylases in an individual or if the protein levels of these BER-related gene products show discordant levels from the mRNA levels. Other collateral events affecting longevity or stability of repair proteins may influence actual outcomes from theoretical. Our analysis demonstrates that targeting DNA repair mechanisms in glioma is likely to require a customized, multi-pronged strategy, personalizable through appropriate profiling of key driver genes of this process.

## OM-049. A WHOLE-GENOME METHYLATION STUDY IN MENINGIOMAS REVEALS PATTERNS OF ABERRANT HYPERMETHYLATION ASSOCIATED WITH CLINICAL AND MOLECULAR AGGRESSIVITY

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Meningiomas are central nervous system tumors that originate from the meningeal coverings of the brain and spinal cord. The majority of meningiomas are benign, but a number of tumors display considerable histological and/or clinical aggressivity, sometimes with unexpectedly high recurrence rates after radical removal. This study aimed to address whether differences in the methylation pattern of these tumors may explain the histological and/or clinical variability observed in meningiomas. We performed a whole-genome methylation study in 38 samples of benign (WHO grade I), atypical (grade II) and anaplastic (grade III) meningiomas (17, 15 and 5 samples, respectively) as well as in 4 non-tumoral meningothelial tissues by using the Infinium Human-Methylation 450 K BeadChip (Illumina, San Diego, CA, USA). Comparison of the methylation patterns between controls and tumors revealed 323 most variant and significant ($\sigma = 0.5$, $q < 0.05$) differentially methylated probes (DMPs), which corresponded to 192 genes. These DMPs allowed the subclassification of tumors according to the differences in the patterns of methylation observed among meningiomas. A subgroup of tumors exhibited an aberrant hypermethylation of most of these DMPs (310, 96%). The group of tumors showing this hypermethylated profile was associated with clinical and molecular features of aggressivity in meningioma. Hypermethylation was observed in 12 of 14 samples obtained from male patients, in 12 of 16 recurrences and in 8 and 4 atypical and anaplastic meningiomas, respectively. In addition, 10 of 13 tumors showing an aggressive gene expression signature previously described by us presented this hypermethylation profile. Our results suggest distinct DNA methylation patterns among meningiomas which may be correlated with different clinical aggressivity. Meningiomas showing aberrant hypermethylated profiles compared to control samples are related with more aggressive tumors.

## OM-050. IDENTIFICATION OF PROGNOSTIC SERUM-ANTIBODIES AGAINST GLIOBLASTOMA-ASSOCIATED ANTIGENS

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A limited number of molecular biomarkers are already successfully used in clinical glioblastoma diagnostics including IDH1 mutation and MGMT hypermethylation. However, they can only be determined by analyzing tissue specimens. Consequently, we are in need for non-invasive prognostic biomarkers that support pre-operative treatment choices. We therefore aimed at screening for prognostic serum antibodies against tumor-associated antigens (TAAs). For that purpose, we designed customized discovery peptide arrays covering the complete linear amino acid sequence of six

known or newly discovered glioblastoma-associated antigens (EGFR, FABP5, GLEA2, MAGEA3, PHF3, TNC). 13mer-peptides were spotted as duplicates with a displacement of 3 amino acids. In order to identify the most prognostic of these 1745 peptides, we hybridized serum of 10 long-term surviving (LTS) and 14 short-term surviving (STS) GBM patients to the arrays. Intriguingly, we observed polyclonal antibodies against TAAs in both LTS and STS patients. Statistical analysis revealed serum antibodies discriminating between LTS and STS patients ($p < 0.03$) against 70 peptides. These were consequently spotted together as a screening peptide array for use in a bigger study sample. A validation study sample consisting of sera from 125 GBMs was used to assess the prognostic value of the 70-peptide chip. Employing a median cutoff for group determination in the survival analysis, we identified 3 prognostic peptides ($p < 0.05$). To further improve the statistical power, we added a second independent multi-center validation study sample ($n = 141$). In the analysis of the combined 266 samples even 13 of the 70 peptides reached significance and turned out to be prognostic ($p < 0.05$). We are currently developing a robust statistical scoring system to improve sensitivity and specificity of our assay. Ultimately, our multi-center serum analysis revealed the prognostic relevance of circulating antibodies and that customized peptide microarrays might comprise powerful tools for non-invasive biomarker discovery.

## OM-051. TRANSCRIPT REARRANGEMENTS INVOLVING CANCER DRIVER GENES IN ADULT LOWER-GRADE GLIOMAS

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The Cancer Genome Atlas (TCGA) is a National Cancer Institute initiative to characterize human cancer genomes and transcriptomes at a single nucleotide resolution. World Health Organization (WHO) grade II and grade III astrocytomas, oligoastrocytomas and oligodendrogliomas are included in the TCGA lower-grade glioma (LGG) effort, tasked with integrative genomic analysis of 500 adult LGGs. Paired-end RNA sequencing can be used to identify transcript fusions that may result in tumorigenic losses or gains of function of cancer proteins. Recurrent tumorigenic transcript fusions and rearrangements have been reported in glioblastoma multiforme (GBM) and pediatric lower-grade glioma (PLGG) tumors, but it is unknown if such events occur in adult LGGs. To address this question, we analyzed RNA sequencing data generated by the TCGA LGG initiative using deFuse and TopHat algorithms, followed by visualizing fusion transcripts in the UCSC genome browser. A deFuse analysis of the transcriptomes of 220 LGGs revealed 25-459 candidate transcript breakpoints per tumor, of which 1-67 involved different chromosomes. Ongoing efforts include computational verification of these events using TopHat and visualization in the UCSC genome browser. Several rearrangements computationally verified to date occurred in IDH-wild-type LGGs and targeted receptor tyrosine kinases, such as EGFR and FGFR3, as previously reported in a subset of GBMs. These fusions were associated with genomic amplifications and had the potential to increase receptor tyrosine kinase activity. We also observed transcript rearrangements involving ATRX in a subset of samples, predicted to result in a loss of function of the protein. Tumors harboring ATRX rearrangements were IDH-mutant and had global expression profiles similar to those of LGGs with ATRX point mutations. This result suggests that transcript rearrangements may provide an alternative mechanism of ATRX inactivation in a subset of LGGs, and that the status of the locus should be carefully investigated in LGG patients.

## OM-052. TUMOR SPECIFIC EPIGENETIC ACTIVATION OF ALTERNATIVE PROMOTERS IN THE BODY OF ONCOGENES

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DNA hypomethylation targets repeat sequences and single-copy loci in glioblastoma (GBM) but the full functional impact of these epimutations remains poorly understood. In the TCGA project, approximately 2% of all CpGs in the methylome were assayed in hundreds of GBM. Here, we assayed 95% of all CpGs with MeDIP-seq and MRE-seq to determine recurring and functionally relevant targets of hypomethylation in a small set of non-G-CIMP primary GBM and compared the results to hundreds of GBM from TCGA. Hypomethylated loci were predominantly intragenic and intergenic and enriched for promoter-associated histone modifications and DNase I hypersensitivity, suggesting tumor-specific hypomethylation of gene regulatory elements within and between genes. Recurrently hypomethylated loci were particularly enriched within a cancer amplicon on chromosome 5p15 that encompasses telomerase reverse transcriptase (TERT). Overall, 76 gene body promoters were recurrently hypomethylated, including TERT, TP73 and GLI3. Furthermore, three out of three novel candidate alternative promoters in gene bodies have significant activity in a promoter luciferase-reporter assay. To further distinguish those hypomethylation events that have a functional consequence, and therefore may be candidate driver epimutations, we performed tissue ChIP-seq on three of our GBM and uncovered H3K4me3 peaks that coincide precisely with loss of DNA methylation at 237 loci, and further, a subset of these genes including GLI3 was associated with increased mRNA level. All but one GBM had TERT 5' promoter mutations, associated with high full-length TERT mRNA and gain of 5' H3K4me3 in the highest expressing tumor. Our findings suggest that gene body promoter hypomethylation, along with other genetic and epigenetic factors, alters the transcriptional landscape of GBM through the activation of a limited number of normally silenced promoters within gene bodies.

## OM-053. GC/MS-BASED METABOLOMIC ANALYSIS OF CEREBROSPINAL FLUID (CSF) FROM GLIOMA PATIENTS

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OBJECTIVE: Metabolomics has recently undergone rapid development, however, metabolomic analysis in cerebrospinal fluid (CSF) has not become a common practice. METHODS: We analyzed the metabolite profiles of preoperative CSF samples from 32 patients with histologically confirmed glioma using gas chromatography/mass spectrometry (GC/MS). We assessed how alterations in the metabolite levels were related to the World Health Organization (WHO) tumor grades, tumor location, gadolinium enhancement on magnetic resonance imaging (MRI), isocitrate dehydrogenase (IDH) mutation status. RESULTS: A total of 61 metabolites were identified in the CSF from glioma patients. The citric acid and isocitric acid levels were significantly higher in the glioblastoma (GBM) samples than in the grades I-II and grade III glioma samples. In addition, the lactic acid and 2-aminopimelic acid levels were significantly higher in the GBM samples than in the grades I-II glioma samples. The CSF levels of the citric acid, isocitric acid, and lactic acid were significantly higher in grade I-III gliomas with mutant IDH than in those with wild-type IDH. The tumor location and tumor enhancement obtained using MRI did not significantly affect the metabolite profiles. Higher CSF levels of lactic acid were statistically associated with poorer prognosis in grade III-IV malignant gliomas. CONCLUSION: Our study suggests that the metabolomic analysis of CSF from glioma patients may be useful for predicting the glioma grade, metabolic state, and prognosis of gliomas.

## OM-054. GLOBAL ANALYSIS OF HISTONE MODIFICATION STATES IN PRIMARY MEDULLOBLASTOMAS REVEALS SUBGROUP-SPECIFIC PATTERNS OF GENE REGULATION

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Recent next-generation genomic studies of medulloblastoma have revealed an unexpected and overwhelming convergence of somatic alterations affecting chromatin-modifying genes. Estimates informed by next-generation sequencing implicate that at least one third of all medulloblastomas have somatic mutations in a chromatin modifier, including those targeting histone methyltransfereses, histone demethylases, and related chromatin modulators that collectively function to influence chromatin conformation associated gene expression states. These mutations occur across all four medulloblastoma subgroups although different sets of genes appear to be selectively altered in a subgroup-specific manner. Despite the abundance of evidence implicating deregulation of chromatin modifiers as a key event in medulloblastoma pathogenesis, the medulloblastoma epigenome remains largely unexplored, and studies cataloguing histone modification states on a genome-wide scale have yet to be reported. To comprehensively investigate the histone code in medulloblastoma and the consequences associated with mutations affecting histone-modifying genes, we have performed ChIP-sequencing on a set of well-characterized primary medulloblastoma specimens. Histone marks examined in this study include the six modifications mandated by the International Human Epigenome Consortium (IHEC), including H3K4me3, H3K9me3, H3K27me3, H3K27ac, H3K4me1 and H3K36me3. Chromatin isolates from primary fresh-frozen tissues representative of each medulloblastoma subgroup were immunoprecipitated with the indicated antibodies and sequenced

with a HiSeq Illumina sequencer to obtain at least 10 million unique reads per ChIP experiment. Peak calling was performed using multiple publicly available tools and data integrated with existing ENCODE data for the same histone marks. Inter-subgroup comparisons of histone modification states revealed a wealth of distinguishing genomic regions that were highly correlated with alternative patterns of gene expression existing between the subgroups. Moreover, integration with existing mutational profiles demonstrated aberrant chromatin states that could be linked to underlying mutations in select chromatin modifiers. Ongoing work will focus on expanding the cohort and integration with all levels of 'omic data.

## OM-055. EXPRESSION OF BRACHYURY IN CHORDOMAS: ASSOCIATION WITH MALIGNANCY

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Chordoma is a rare, clinically malignant bone tumor characterized with invasiveness, repeated recurrence, and poor prognosis. Chordoma is thought to arise from remnants of the notochord, and recently studies have revealed that chordoma specifically expresses brachyury, which is a transcription factor associated with differentiation of the notochord. However, the clinical implication of brachyury remains unclear. In this study, we investigated the relationship between the malignancy of chordoma and the expression of brachyury. Twenty-seven skull base chordomas, that had been resected at our institution, were analyzed for brachyury expression by the real-time PCR. Brachyury expression was positively correlated with Ki-67 labelling index, which was thought to be associated with tumor doubling time; brachyury expression in the tumors with Ki-67 index over 6% was significantly higher than that of the others ($P = 0.0014$). The detailed analysis on recurrent tumors revealed that brachyury expression was lost in some cases compared with that of the original tumors; sarcomatous transformation was identified in one recurrent tumor. We concluded that brachyury expression was correlated with aggressiveness of chordoma. Further analysis on genetic and/or epigenetic changes in the cases showing loss of brachyury expression remained to be investigated.

## OM-056. HUMAN NON-GCIMP GLIOBLASTOMA SUBTYPES EVOLVE FROM A COMMON PRONEURAL-LIKE PRECURSOR GLIOMA

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Glioblastomas (GBMs) are the most common and malignant form of the central nervous system neoplasms. Deep molecular analysis of GBMs has subdivided them into 4 transcriptomal classes denoted proneural (PN), mesenchymal (MES), classical (CL), and neural (NL). These subclasses can be associated with canonical mutations such as PDGFRα amplification in PN-GBM, loss of NF1 in MES-GBM, and amplification of EGFR in CL-GBM. Within the PN-GBM subgroup are those tumors that are mutant for IDH1/2 and have the methylator phenotype denoted by GCIPM. However, in some cases the subdivision is blurred in that a sample might show expression patterns of more than one subtype and the significance of the relation between the subtypes are not still established well. So to understand the relationships between the human non-GCIMP GBM subgroups, we performed computational analysis of human genomic data to predict the temporal sequence in which the driver events arise during tumorigenesis. The order of evolutionary events for non-GCIMP GBM is 1) chr 7 gain and loss of chr 10, followed by 2) CDKN2A loss and/or TP53 mutation, and 3) alterations canonical for specific subtypes such as NF1 loss or focal amplification of PDGFRα or EGFR. We then developed a computational methodology to identify the drivers of broad copy number changes, identifying PDGF-A (chr 7) and PTEN (chr 10) as driving the initial non-disjunction events. These predictions were validated using mouse modeling, showing PDGF-A is sufficient to induce PN-like gliomas that are enhanced by loss of Ink4a-Arf, Tp53 or Pten. Additional Nf1 loss converts PN to the MES subtype. Our findings suggest non-GCIMP GBMs arise as, and evolve from, a common proneural-like precursor and provide insight into therapeutic strategies targeting late events in GBM evolution because the GBMs are likely to already represent a lethal disease prior to acquiring these mutations.

## OM-057. ANALYSIS OF IDH1 MUT EXPRESSION IN HUMAN ASTROCYTOMAS BY RT-PCR

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IDH1 mutations are frequent somatic mutations in low grade glioma (LGG) as well as in secondary high grade glioma (HGG). These mutations result from heterozygous mutations that affect codon 132. To date, gene sequencing is the "gold standard" in analyzing the IDH mutational status in glioma patients. Recently, measuring the IDH1 mut level by real time PCR was introduced as an alternative and even more sensitive method. Therefore, we set out to investigate if the expression of IDH1 mut on RNA level correlates with the course of disease and, thus, might be used as a biomarker for disease progression. 71 tumor samples from LGG and HGG patients were divided into 7 subgroups based on tumor grading (control brain tissue, diffuse glioma, anaplastic glioma, sGBM +/- Cx, pGBM +/- Cx). After RNA isolation quantitative expression of IDH1 mRNA was assessed using real-time PCR. IDH mutational status was verified by gene sequencing and Immunohistochemistry. We found a high sensitivity (96%) and specificity (95%) of RT-PCR in analyzing the IDH1 mutational status. Furthermore, we were able to define a cut-off value (0.111) above which sensitivity and specificity were 100%. In our quantitative analysis of progressive glioma we found an increasing expression of IDH1 mutation from LGG to HGG (WHO II° Mean $\pm$ SEM: $0.09 \pm 0.01$; WHO III°: $0.17 \pm 0.03$; sGBM: $0.37 \pm 0.03$). Moreover, we observed lower IDH1 mutation expression in patients having received prior chemotherapy (sGBM - Cx: $0.37 \pm 0.03$ vs. sGBM + Cx: $0.25 \pm 0.06$). Our results confirm RT-PCR as a useful tool in analyzing the IDH1 mutational status in glioma patients. Furthermore, we did not only observe an influence of prior chemotherapy on IDH1 mut expression but also an increase of expression in progressive glioma. Therefore, quantification of IDH1 mutation expression could be a potential candidate as a biomarker for disease progression.

## OM-058. BIOMARKERS INDICATIVE OF TENDENCY TO RECUR ON MENINGIOMAS: AN IMMUNOHISTOCHEMICAL AND MOLECULAR STUDY RELATED TO CLINICAL DATA AND FOLLOW UP

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Meningiomas constitute approximately one-third of primary CNS tumors. Most of them are classified as Meningioma (grade I) and are treated by surgical resection. However other different types of meningioma exist: atypical meningioma (grade II) and anaplastic meningioma (grade III), with poor outcome. Aim of this study was to investigate biomarkers implicated in tumorigenesis compared to clinical data, looking for prognostic factors suggestive of tendency to recur and potential therapeutic targets. We performed a retrospective study on 150 patients with 15-10 years follow up for meningiomas grade I-II. They were 50 meningiomas (grade I) without recurrence, 40 meningiomas (grade I) with recurrence, 40 atypical meningiomas and 20 anaplastic meningiomas. We investigated biomarkers implicated on different pathways: i) Angiogenesis (Vascular Endothelial Growth Factor (VEGF), angiogenesis promoter; Endoglin (CD105) marker of activated endothelial cells). ii) Receptor tyrosine kinases (Platelet-Derived Growth Factor Receptor a and b (PDGFR), implicated in tumorigenesis, related to expression of c-Kit; Fibroblast Growth Factor Receptor (FGFR)). iii) Invasion (Matrix-metalloproteinase (MMP9, 2, 3); cell adhesion molecules (β-catenin and E-cadherin). iiii) Proliferation (Cyclin-Dependent Kinases Regulatory Subunit 2 (CKS2) regulating metaphase/anaphase transition, down-regulating the gene codifying Leptin Receptor (LEPR2)). We analysed the expression of biomarkers using immunohistochemistry, Western Blot and RT-PCR. The results were correlated with clinical data. Our results suggest high frequency of recurrence on cases with high expression of PDGFR, c-Kit, MMP9, CKS2. In particular, increasing expression of PDGFR was observed related to the grade. c-Kit immunohistochemical expression was present on about 20% of cases with worse prognosis. MMP9 seems to be a negative prognostic factor independently to the grade. CKS2 expression was related to recurrences. In atypical meningiomas β-catenin seems related

to better prognosis. In conclusion, the different expression profiles of biomarkers in meningiomas related to clinical data, may have a role as new prognostic markers and potential therapeutic targets.

### OM-059. CHROMOSOMAL IMBALANCE AND SURVIVAL IN PATIENTS WITH ASTROCYTOMA

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Brain tumors are the most frequent solid tumor in children with a 5-year survival rate of 60%. The cytogenetics applied to these tumors has revealed that molecular alterations may lead to the development of progression and lack of response to treatment. The association between the chromosomal imbalance and the survival rate will assess the prognosis of these tumors, allowing us to divide them into groups and provide specific and individualized treatment according to the chromosomal alterations that they present. OBJECTIVE: To determine the association between chromosomal imbalances, patient survival and clinical characteristics. MATERIALS AND METHODS: Records of patients diagnosed with astrocytoma were reviewed from 1995 to 2005 at the Oncology Service in the Hospital de Pediatria in Mexico City. Age, gender, location, grade of resection, histology and treatment were documented. Comparative Genomic Hybridization (CGH) analysis were performed in tumor samples taken from paraffin blocks to identify chromosomal gains and losses. RESULTS: 35 patients were studied with a 5-year survival rate of 62.9%. 31 (88%) had alteration(s) in chromosomes 1, 5, 9 and 18; and 23 (65.7%) presented gains or losses in chromosome 18. According to the histology of each sample, 77% of the astrocytomas (diffuse and pylocitic) had alterations in chromosome 9 and 88% of anaplastic astrocytomas presented alterations in chromosome 18. The survival rate of patients with no alterations in Cr 1 was 72.2% compared with 52.9% with patients who presented alterations. For patients with alterations in Cr 5 the survival rate was 59% versus 61% for those who did not. Finally, for patients with chromosomal alterations in Cr 18 the survival rate was 58.3% versus 62% for those who did not. The results suggest that patients with chromosomal imbalances had a lower survival rate. The associations in this study are not statistically significant due to the small sample.

### OM-060. $O^6$-METHYLGUANINE-DNA METHYLTRANSFERASE (MGMT) ACTIVITY IN ANAPLASTIC OLIGODENDROGLIAL TUMORS: ASSOCIATIONS WITH ALKYLATING AGENT RESPONSE, 1p19q DELETION AND *MGMT* PROMOTER METHYLATION

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The role of the DNA repair protein $O^6$-methylguanine-DNA methyltransferase (MGMT) in alkylator resistance in high-grade gliomas to continues to be the focus of intensive investigation. While *MGMT* promoter CpG methylation and MGMT protein expression have been associated with alkylator response, there few studies documenting the contribution of MGMT enzymatic activity to treatment response, especially for anaplastic oligodendroglial (AO) tumors. Our hypothesis is that MGMT activity in AO is inversely associated with clinical response following alkylator therapy. We further hypothesize that low MGMT activity underlies the better therapeutic outcome characteristic of AO with 1p19q deletions and/or promoter methylation. To address this hypothesis, we assayed MGMT biochemical activity 42 AO with known deletion and methylation status and examined the relationship between activity and PFS after alkylator therapy using Cox proportional hazards regression analysis. We found that activity was significantly lower in tumor with deletions tumors ($3.8 \pm 2.1$ *vs.* $11 \pm 11$ fmol/$10^6$ cells; $P \leq 0.02$) or promoter methylation ($4.3 \pm 2.3$ *vs.* $15 \pm 13$ fmol/$10^6$ cells; $P \leq 0.03$). Univariate Cox regression analysis revealed that tumors with greater than median MGMT activity (4.7 fmol/$10^6$ cells) had greater risk of progression (HR = 2.9; $P \leq 0.02$) and median PFS (11.5 *vs* 46 months; $P \leq 0.007$). Importantly, tumors with greater than median activity were less likely to harbor 1p19q deletions (20% *vs* 64%; $P \leq 0.04$) or display promoter methylation (31% *vs* 92%; $P \leq 0.003$). Our findings indicate that MGMT activity promoters clinical resistance to alkylators in AO and that the low MGMT activity may underlie the better clinical response that accompanies deletion at 1p19q or promoter methylation. Our results support the use of MGMT inhibitors to improve treatment outcome in AO and suggest that MGMT activity may have utility as a marker to direct treatment.

### OM-061. IDENTIFICATION OF DIFFERENTIALLY REGULATED miRNA IN THE BLOOD OF SHORT- AND LONG-TERM PCNSL SURVIVORS BY NEXT GENERATION SEQUENCING

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Primary CNS lymphoma (PCNSL) remains a therapeutic challenge in neuro-oncology. Several analyses have repeatedly demonstrated that higher age and low Karnofsky Performance Status (KPS) are associated with shorter overall survival (OS). microRNA (miRNA) are small RNA molecules involved in the posttranscriptional gene regulation. They have also gained increasing interest as potential biomarkers in the blood of cancer patients. Within this study we aimed at defining miRNA expression profiles in the blood of PCNSL short- (STS) and long-term survivors (LTS) to assess their potential as novel prognostic markers. All blood samples were collected at the time of enrolment in the G-PCNSL-SG1 trial, a large randomized phase III study which assessed the role of consolidating whole brain radiation therapy (WBRT) in PCNSL patients. We set up 2 cohorts of 20 patients with STS patients having a median OS of 3 months compared to 55 months for the LTS group. Both cohorts were balanced for median age and KPS: 64 years and 70% for STS compared to 62 years and 70% for LTS. miRNA was extracted from blood samples and analyzed using Next Generation Sequencing. The biostatistical analysis revealed a differential regulation of several miRNAs in the 2 cohorts. An in silico enrichment analysis demonstrated that 9 deregulated miRNAs are known to be onco-miRNAs representing a significant enrichment for these markers ($p < 0.001$). Real-time PCR was used to confirm the deregulation of the most promising candidate miRNAs as well as novel short RNA molecules with putative miRNA function not described before. Based on the results of this study, blood-derived miRNA profiles warrant further exploration as a prognostic tool in PCNSL patients.

### OM-062. IDENTIFICATION OF DISTINCT SUBGROUP OF GLIOMA AND NOVEL GENES RELATED TO MALIGNANT PROGRESSION BY GENOME-WIDE METHYLATION ANALYSIS

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INTRODUCTION: Recent genome-wide methylation profiling in gliomas revealed a subgroup which showed an overall increase in DNA methylation at CpG sites (CpG island methylator phetnotype in glioma; G-CIMP). However, impact of G-CIMP on pathogenesis and progression of glioma remained to be completely elucidated. METHODS: We performed genome-wide methylation analysis of 100 glioma samples including 14 paired samples of primary and recurrent gliomas and 4 normal brains using the Illumina Infinium HumanMethylation450 platform. We further validated the results on an external dataset from the TCGA. RESULTS: In addition to the identification of G-CIMP, we identified one distinct methylation cluster within G-CIMP tumors. Remarkably, tumors in this cluster consisted of G-CIMP positive glioblastomas and had a different methylation profile compared to their original lower grade samples. Conversely, some other secondary glioblastomas did not show the change in methylation profile, indicating a different pathogenesis of malignant progression. These clusters were also identified by a clustering together with an external dataset from the TCGA. We found these differentially methylated or unmethylated genes showed distinct patterns of specific histone modifications in human embryonic stem cells and neuronal cells, suggesting the possibility that epigenomic statuses of cancer progenitor cells could affect the susceptibility of aberrant methylation during malignant progression of glioma. Integration of the gene expression and DNA methylation identified novel genes with both methylation and gene expression changes in the former cluster. The selected genes included oncogenes that could play an important role for proliferation or invasion. The genes were successfully validated by

quantitative PCR and MassARRAY. CONCLUSIONS: We first showed the apparent change in methylation profile after malignant progression of glioma and subclassified CIMP positive glioblastoma into two groups. Additionally, we identified novel genes that could play an important role in malignant progression.

### OM-063. PRECISE RECONSTRUCTION OF AMPLIFIED REGIONS IN GLIOBLASTOMA MULTIFORME USING HIGH-THROUGHPUT SEQUENCING DATA FROM THE CANCER GENOME ATLAS REVEALS ONCOGENIC DOUBLE MINUTE CHROMOSOMES

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The true power of tumor DNA sequencing comes from thoughtfully integrating the various genomic perturbations revealed by analyzing the sequencing data, including structural rearrangements, copy number, and mutations, to form a more complete understanding of the tumor genome. We can then begin to piece together the events characterizing the genesis and subsequent development of individual tumors. We developed methods to compute copy number, detect structural variants, and then synthesize these data to locally reconstruct highly rearranged regions of the tumor genome with high precision from standard short read, paired-end sequencing datasets. We find that circular assemblies are the most parsimonious explanation for a set of highly amplified tumor regions in a subset of glioblastoma multiforme (GBM) samples sequenced by The Cancer Genome Atlas (TCGA) consortium, indicating the possible existence of double minute chromosomes (DM) in these tumors. Further, we find that some samples harbor multiple circular amplicons and in some cases further rearrangements occurred after the initial amplicon-generating event. Fluorescence in situ hybridization (FISH) analysis confirms the presence of DMs in two of these samples. Analysis of the gene content of these assemblies helps identify likely driver oncogenes for these amplicons. RNA-seq data available for one DM provides further support of our local tumor genome assemblies, and identifies the birth of a novel exon made possible through rearranged sequences present in the DM. Consistent with previous estimates, in a larger set of GBM tumors with exome sequencing data, our method finds evidence for oncogenic DMs in over 20% of samples.

### OM-064. THE COMBINATION OF INTERLEUKIN-10 AND BETA2-MICROGLOBULIN IN CEREBROSPINAL FLUID IS A USEFUL BIOMARKER IN IMMUNOCOMPETENT PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL)

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OBJECTIVE: The diagnosis of primary central nervous system lymphoma (PCNSL) is often difficult. The objective of this work is to evaluate the diagnostic value of interleukin-10 (IL-10), IL-6, soluble IL-2 receptor (sIL-2R), and beta2-microglobulin (b2-M) levels in the cerebrospinal fluid (CSF). METHODS: (1) Case-control study: the CSF levels of IL-10, IL-6, sIL-2R, and b2-M in 66 patients with intracranial tumors (PCNSLs: 26 cases) were analyzed. (2) Prospective study: To verify the diagnostic value, patients with brain lesions that were preoperatively suspected of being PCNSLs on MRI were prospectively analyzed and CSF IL-10 and b2-M were measured from December 2010 to December 2012. RESULTS: (1) Case-control study: In PCNSLs, median CSF levels of IL-10, IL-6, b2-M, and sIL-2R were 27 pg/ml, 5.4 pg/ml, 4084 μg/l, and 100 U/ml, respectively. On the other hand, in other brain tumor types, the median levels of IL-10, IL-6, b2-M, and sIL-2R were 2.0 pg/ml, 2.7 pg/ml, 1200 μg/l, and 50 U/ml, respectively. Median CSF levels of IL-10, b2-M, and sIL-2R were significantly higher in the patients with PCNSL ($p < 0.001$). (2) Prospective study: Fifty-five patients were enrolled. Fourteen patients were diagnosed as PCNSL. In PCNSLs, median CSF levels of IL-10 and b2-MG were 63 pg/ml and 4325 μg/l, respectively. On the other hand, in other brain tumor, the median CSF levels of IL-10 and b2-M were 2.0 pg/mL and 1150 μg/l, respectively. The CSF IL-10 and b2-MG levels were significantly increased in the patients with PCNSLs ($p<0.001$). The sensitivity and specificity of IL-10 (>9.5 pg/ml) were 78.0% and 100%, respectively. Also, the sensitivity and specificity of b2-M (>2676 μg/l) were 78.0% and 100%, respectively. IL-10 (>9.5pg/ml) or b2-M (>2676 μg/l) showed quite high sensitivity and specificity for the diagnosis of PCNSL. CONCLUSION: The combination of CSF IL-10 and b2-M were quite useful diagnostic biomarker for PCNSL.

### OM-065. MicroRNA PROMOTER METHYLATION AND EXPRESSION IN ANAPLASTIC GLIOMA

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To improve the prognosis and treatment of gliomas, we took advantage of the data and tissues of the Neurooncology Working Group of the German Cancer Society (NOA) Study-04. Initially, epigenetic regulation of miRNA was assessed with a methyl-CpG-immunoprecipitation (MCIp) screen on anaplastic glioma patient samples (n = 4; healthy controls n = 1) to determine methylated CpG DNA stretches and an H3K4me3 chromatin immunoprecipitation (ChIP) screen to assess active miRNA promoter regions in cell lines of different origins (n = 6). Overlaying results of these screens revealed 14 miRNA promoter regions, which were validated on a set of 100 anaplastic glioma patient samples from the NOA-04 study trial cohort and additional 80 anaplastic glioma samples provided by the German Glioma Network. The MassARRAY methylation levels of these genomic regions were statistically correlated with progression-free (PFS) and overall survival. A Cox regression model yielded three differentially methylated miRNA promoter regions, which significantly influenced survival (mir-155; mir-210; mir-335). In each case, higher methylation was associated with a longer survival. Moreover, there was a correlation with an isocitrate dehydrogenase (IDH) mutation and $O^6$-methylguanine-methyltransferase (MGMT) promoter methylation for these candidates. However, mir-155 also had an independent prognostic impact for PFS. Additionally, expression levels of mir-155 correlated with the miRNA promoter methylation in 12 anaplastic glioma patient tissues. This was confirmed by The Cancer Genome Atlas (TCGA) data set of anaplastic gliomas (n = 90), where a higher mir-155 promoter methylation was associated with a lower mir-155 expression and both were correlated with a prolonged survival. We conclude that promoter methylation of mir-155, mir-210 and mir-335 is of prognostic relevance in anaplastic gliomas. In case of mir-155, the methylation is additionally inversely correlated with the expression and independent of IDH mutation, which makes it an interesting candidate for further analysis.

### OM-066. LOW LEVELS OF HUMAN CYTOMEGALOVIRUS INFECTION IN GLIOBLASTOMA MULTIFORME ASSOCIATES WITH PATIENT SURVIVAL; A PROSPECTIVE STUDY

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BACKGROUND: Over 90% of GBM tumors have been shown to be infected with human cytomegalovirus (HCMV). In this study, we evaluated whether there was an association between the grade of HCMV infection and long-term survival in GBM patients. MATERIAL AND METHODS: Brain tumor tissue sections from consecutive GBMs patients who were operated at the Karolinska University Hospital from 2003 were analysed (n= 250). HCMV infection grade was determined by estimation of the number of HCMV positive cells (scored negative or grade 1-4) by immunohistochemistry in tumor tissue specimens. Using Chi-Square test and logistic regression analysis, we analyzed whether there was an association between long-term survival and HCMV low-grade infection or other clinical parameters known to be associated with prolonged survival of GBM patients; age under 50 years, radical surgery or low recursive partition analysis (RPA) and MGMT status. RESULTS: HCMV infection was detected in tumor samples from 249 of 250 patients (99%). Among patients surviving > 18 months, HCMV infection grade 1 in the GBM tumor was predominant. Forty percent of GBM patients who lived > 18 months had low-grade HCMV infection while only 8% GBM patients who lived < 18 months did. In the first 75 cases, multiple logistic regression

analyses yielded an odds ratio estimate of 6.61 with 95% confidence interval (1.36-32.1) (*p .019*) for low grade HCMV after adjustment for RPA class, radical surgery, and age. The remaining data assessment will be completed and presented at the meeting. CONCLUSION: We previously that reported that low-grade HCMV infection was strongly associated with long-term survival in GBM patients in a case-control study of 75 patients. This larger prospective study of patients treated at a single institution confirms the previous data. As a consequence, CMV analysis by immunohistochemistry could be use as a prognostic marker in glioblastoma.

## OM-067. A MOLECULAR PREDICTOR OF RESPONSE TO BEVACIZUMAB BASED ON ANALYSIS OF RTOG 0825, A PHASE III TRIAL COMPARING CHEMORADIATION WITH AND WITHOUT BEVACIZUMAB IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA

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RTOG 0825 evaluated the addition of bevacizumab (BEV) to standard chemoradiation in the treatment of glioblastoma (GBM) and included tissue collection for molecular stratification and analysis. We hypothesized a molecular signature might predict for response to BEV. Sufficient tissue for molecular analysis was available for 650 registered, eligible patients. Molecular stratification for mesenchymal enrichment was performed by real-time PCR prospectively and an expanded set of mesenchymal genes was evaluated retrospectively on a subset of cases. Additional whole genome expression profiling (GEP) was performed. Predictive models were evaluated for their ability to predict overall survival (OS) in a randomly selected set from the BEV arm. Cox regression was performed to adjust for prognostic factors and treatment arm interaction. Unsupervised clustering of GEP data was used to identify molecular subsets. We observed a significant association between favorable molecular stratification signature and improved PFS and OS in the BEV arm ($p = 0.035$ & $p = 0.034$, respectively). Based on the association between mesenchymal expression and outcome in the BEV arm, we sought to optimize a predictor using an expanded set of mesenchymal genes. Unbiased gene selection followed by predictive modeling identified a multigene glioma BEV response predictor (gBRP) in the BEV arm ($p < 0.0001$/HR 0.28 for OS and $p = 0.0005$/HR 0.41 for PFS) after adjusting for prognostic factors (MGMT, RPA), with no significant role in the standard arm. To further support the association of MES enrichment and poor outcome in the BEV arm, we performed GEP. Unsupervised clustering identified tumors with MES enrichment that correlated to unfavorable gBRP class ($p = 0.0486$). In conclusion, we developed a gBRP that identifies subsets of patients with differential response to BEV treatment that may serve to identify patients with newly diagnosed GBM who benefit from BEV. Supported by RTOG U10-CA21661, CCOP U10-CA37422 and P50-CA127001 from the NCI, and Genentech.

## OM-068. microRNA 371-373 AND 302A IN CEREBROSPINAL FLUID ARE POTENTIAL TUMOR-DERIVED BIOMARKERS FOR INTRACRANIAL GERM CELL TUMORS

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BACKGROUND/AIMS: Intracranial germ cell tumors (iGCTs) are subclassified into germinoma and nongerminomatous GCT (NGGCT). Although beta human chorionic gonadotropin and alpha fetoprotein in either serum or cerebrospinal fluid (CSF) have been used as biomarkers for NGGCT, there is no reliable biomarker for germinoma. Recent study of microRNA (miRNA) expression profiling in systemic malignant GCT tissues showed miR-371-373 and mir-302-367 clusters to be the most over-expressed miRNAs and some of these miRNAs are differentially expressed in different subtypes of GCT. METHODS: Total RNA was extracted from 100 microliters of CSF and expression of miR-371-373 and mir-302-367 clusters was measured by TaqMan® Array MicroRNA TLDA cards. Delta threshold cycle (CT) was calculated by using spike-in ath-miRNA-159a as control. RESULTS: A total of 32 CSF samples from 22 iGCT patients (12 germinomas and 10 NGGCTs) were analyzed. MiR-371-3a, miR-372, miR-373 and miR-302a# showed significant over-expression (log2 fold change 4.72 - 8.87) in GCT patients compared with control. Expression of these miRNAs was significantly higher in pre-treatment CSF than those collected during or post-treatment. The expression of miR-373 was significantly higher in CSF of germinoma than NGGCT. When receiver-operating characteristics (ROC) curves were compared, area under the curve (AUC) was highest for miR-372 (0.92) to separate between GCT and other disease with accuracy of 91.7% (81.3% sensitivity; 100% specificity). AUC of ROC curve was 0.67 for miR-373 to separate between germinoma and NGGCT with accuracy of 81.3% (80% sensitivity; 83.3% specificity). CONCLUSION: We demonstrate for the first time the potential utility of miRNAs in CSF as diagnostic biomarkers for subclassifying intracranial GCTs and to monitor treatment response.

## OM-069. A SUBSET OF THE PERIOSTIN GENE NETWORK IS RELATED TO SURVIVAL AND RECURRENCE IN GLIOMAS

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BACKGROUND: Gliomas are the most common primary brain tumors and they often recur locally. Recently, the matricellular protein periostin (POSTN) has been shown to be associated with poor prognosis in GBM. We hypothesize that a subset of genes associated with POSTN may also play a role in glioma progression and recurrence. Utilizing a public dataset of 23 paired samples of primary high-grade gliomas and recurrences, we identified such a subset and validated it in an external dataset and by qPCR in an independent set of recurrent gliomas. METHODS: We used the gene expression data from 559 GBM patients in the Cancer Genome Atlas (TCGA) to build a network expression model of POSTN. We modeled the gene expression changes seen in 23 paired recurrent samples included in GSE4271 utilizing Significance Analysis of Microarrays (SAMr). The intersection of these models was used to build a genetic classifier of aggressive disease. Validation was performed in 419 patients from the REMBRANDT dataset and with qPCR in an independent set of 15 paired recurrent glioma samples. RESULTS: A total of 7550 genes were significantly changed between the 23 paired samples at time of recurrence. Only 10 of these genes were also highly correlated with POSTN at diagnosis. A predictive model from these 10 genes applied to the REMBRANDT dataset demonstrated significantly different survival between classes (log rank $p < 0.0005$) and qPCR of a subset of these genes showed significant correlation with POSTN in recurrent gliomas. Gene set enrichment reveals these genes are related to integrin interactions, invasive breast cancers, and mesenchymal transition. CONCLUSIONS: We have developed a gene network model of POSTN to investigate genes related to recurrence in glioma. Selected genes are related to invasion and stemness and are potential targets for further treatment, warranting further study of the signatures of glioma recurrence.

## OM-070. IDENTIFICATION OF ANAPLASTIC OLIGODENDROGLIOMA PATIENTS BENEFITTING FROM ADJUVANT PCV CHEMOTHERAPY USING THE ILLUMINA PLATFORM: A FURTHER REPORT FROM EORTC STUDY 26951

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INTRODUCTION: Adjuvant procarbazine, CCNU and vincristine chemotherapy (PCV) improves Overall Survival (OS) in 1p/19q co-deleted

oligodendrogliomas. Previous analyses of EORTC study 26951 suggested CpG-Island Hypermethylated Phenotype (CIMP+) to be a promising biomarker. We further explored this on a larger series using formalin fixed paraffin embedded (FFPE) tumor material, and compared this to the predictive value of 1p/19q status, IDH and MGMT. METHODS: Methylation profiles were assessed using the Infinium HumanMethylation450 BeadChip (Illumina). MGMT promoter methylation was re-assessed with a logistic regression model (MGMT-STP27) using probes cg1243587 and cg12981137 that correspond to area's of the promoter correlated to MGMT protein expression (Bady et al, Acta Neuropathol 2012;124:547-60). Previously, MGMT promoter methylation had been assessed using methylation specific multiplex ligation-dependent probe amplification (MS-MLPA). RESULTS: Material of 115 patients was available for methylation profiling. Although prognostic factors were well balanced, overall survival (OS) within the RT-only treatment arm of included patients was worse compared to OS in patients not included. For 91 cases, information on all biomarkers was present. In multivariate analysis, 1p/19q co-deletion and CIMP status were independent prognostic factors. Although 1p/19q status and IDH mutational status identify subgroups with more benefit of PCV chemotherapy, tests for interaction remain negative (p 0.25 and 0.33 respectively); MS-MLPA had no impact on treatment effect (p = 0.70). CIMP status was of borderline predictive significance (p= 0.07), and MGMT-STP27 was of statistical significance (p = 0.003; HR unmethylated 1.61, 95% CI [0.71, 3.66], HR methylated 0.37, 95% CI 0.23, 0.61]. Exploratory analysis revealed a subset of 20 CpG sitesthat distinguished between positive and negative OS effects of the additon of PCV. CONCLUSION: CpG site methylation assessment with the HM450 BeadChip appears the most informative tool for identifying grade III glioma patients benefitting from the addition of PCV to RT. Validation is required.

## OM-071. COMPREHENSIVE MGMT AND MISMATCH REPAIR ANALYSIS IN PAIRED PRIMARY AND RECURRENT GLIOMAS REVEALS EVOLUTIONARY AND TEMOZOLOMIDE-INDUCED CHANGES DURING TUMOR PROGRESSION

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The wide range in duration of response to temozolomide (TMZ) in patients with MGMT methylated gliomas illustrates the need to improve use of predictive biomarkers. Mismatch repair (MMR) plays a central role in tumor response to TMZ, particularly when repair by MGMT is compromised. Here we explored factors that might better predict response to TMZ, including intratumoral heterogeneity, tumor evolution, and combined genetic and epigenetic mechanisms acting upon MGMT. In a cohort of primary low-grade gliomas and their recurrences we assessed MGMT enhancer methylation from bisulfite sequencing and Infinium 450K arrays, MGMT and MMR copy number and mutation status from exome sequencing, and extracted MGMT expression from RNA sequencing. A common finding was gain of methylation over time in the MGMT enhancer. A diverse spectrum of patient-specific changes to MGMT genetic and epigenetic status was also discovered. In one patient, subclonal deletion of MGMT in the primary tumor emerged as the dominant clone in the recurrence, coincident with an increase in enhancer methylation and a decrease in MGMT expression, suggesting biallelic inactivation. We looked for intratumoral heterogeneity of methylation across the MGMT enhancer region. In one of three patients analyzed, methylation levels ranged from 24% to 84% in distinct pieces of the same surgery. Four of seven patients treated with TMZ acquired MMR mutations at recurrence, each bearing the signature of TMZ-induced mutagenesis and recurring as GBM. In contrast, in the group of fourteen untreated patients MMR gene mutation was detected in only one primary tumor. We conclude that 1) MGMT methylation exhibits significant intratumoral heterogeneity in some but not all patients tested, increased sampling may enhance clinical value of MGMT, and 2) ongoing evolution of the genetic and epigenetic status of MGMT and MMR repair, induced spontaneously or directly by TMZ, may result in tailored use of these markers.

## OM-072. LANDSCAPE OF CHROMOSOMAL COPY NUMBER ABERRATIONS (CNAS) IN GLIONEURONAL TUMORS (GNTS): RELATIVELY FREQUENT GAIN OF CHROMOSOME 5 AND/OR 7; CHROMOTHRIPSIS IN SUBSET OF CASES

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INTRODUCTION: GNTs are infrequent, generally benign, diagnosed during childhood and associated with medically intractable epilepsy. Sporadically, pathological progression to a high-grade glioma is observed. Current knowledge on molecular characteristics is limited. A subset of GNTs has been reported to carry the BRAFv600E mutation. AIM: To explore the landscape of CNAs in a large cohort of GNTs and to correlate those to histological and clinical characteristics. MATERIALS AND METHODS: We screened a cohort of 131 GNTs diagnosed in children and young adults (dysembryoplastic neuroepithelial tumors (DNTs) n = 67, ganglioglioma (GG) n = 52, desmoplastic infantile ganglioglioma (DIG) n = 4, gangliogliomа grade III (GG-III) n = 6, papillary GNT (PGNT) n= 1). DNA from formalin fixed paraffin embedded (FFPE) tissue was used to generate chromosomal copy number profiles with genome wide shallow Massively Parallel Sequencing (MPS). RESULTS: In about half of our cohort no CNAs were detected. In the other samples a variable pattern of CNAs was identified, gains of chromosomes 5 and 7 being the most frequent CNAs in DNTs. In four tumors (2 DNTs, 1 GG WHO I, and 1 GG WHO III) somatic intra- and/or interchromosomal chromothripsis was detected in chromosomes 7 and 12. This was not associated with shorter survival in these four patients. CONCLUSION: Our study provides comprehensive information on the landscape of CNAs in a large series of GNTs. Moreover, we demonstrate for the first time the occurrence of somatic chromothripsis in benign tumors. Currently, we are investigating the molecular mechanisms that drive this 'catastrophic genomic event' as well as the clinical significance of (the absence of) other chromosomal aberrations in GNTs.

## OM-073. GENOMIC CHARACTERIZATION OF SPATIAL AND TEMPORAL HETEROGENEITY OF GLIOBLASTOMA MULTIFORME

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The extent to which intratumoral genomic heterogeneity in glioblastoma (GBM) represents a stable equilibrium or a snapshot of a dynamic process remains unknown. To better understand the selective pressures that contribute to tumor progression and therapeutic resistance in GBM, we performed exome sequencing, transcriptome, copy number and methylation profiling of primary and first tumor recurrence of thirteen GBM patients. Multiple spatially distinct samples were available for six primary and eight recurrent cases, allowing information regarding intratumoral heterogeneity to be incorporated. We observed a 40% to 80% overlap between mutations from different geographical regions in primary tumors. Mutations shared between both

primary tumor sites had greater likelihood to also be observed in the tumor recurrence and thus possibly reflect clonal sequence variants. Of primary tumor driver mutations, in genes such as TP53 and PIK3CA, 77% were also found in the post-treatment tissue, whereas this true in only 58% for non-drivers. Recurrent tumors harbored approximately 50% more mutations than primaries and showed an increased relative fraction of A > C and A > G substitutions. Recurrent tumors showed a significantly increased number of anti-apoptosis mutations compared to primaries and we did not observe an association between radio-/cytotoxic therapy and hypermutation. To account for the detection sensitivity of exome sequencing, we employed very deep sequencing (>1,000X) to test whether recurrence-unique mutations can be found at very low frequencies in the primary tumors, and found that recurrence-unique mutations were in majority acquired during post-diagnosis, during tumor regrowth. Integration of various genomic data types revealed that the intratumoral heterogeneity of primary tumors is higher and more variable than that of recurrent tumors, and that subclonal tumor cell populations could be distinguished in the majority of primary and post-treatment samples. Analysis of spatially and temporally distinct samples highlights the genomic heterogeneity of GBM and provides novel avenues for therapy development.

## OM-074. AN INTEGRATED GENOMIC ANALYSIS OF INDIVIDUAL PATIENTS WITH GLIOBLASTOMA REVEALS PREVIOUSLY UNRECOGNISED LEVELS OF INTRA-TUMOUR HETEROGENEITY THAT REFLECTS CANCER EVOLUTIONARY DYNAMICS

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BACKGROUND: Glioblastoma (GB) is the most common and aggressive primary brain malignancy, with poor prognosis and a lack of effective therapeutic options. Accumulating evidence suggests that intra-tumor heterogeneity is likely to be the key to understanding treatment failure. However, the extent of intra-tumor heterogeneity as a result of tumor evolution is still poorly understood. METHODS: Using a Fluorescence-Guided Multiple Sampling (FGMS) approach we obtained between 4 and 6 tumor fragments from spatially distinct areas of the visibly fluorescent tumor mass that were at least 10mm apart in each of 11 GB patients. Samples were subject to genome wide copy number analysis on the Affymetrix SNP6 platform, gene expression profiling on the Illumina HT12 platform and methylation molecular clock analysis using the Roche 454 GS Junior system. RESULTS: We present an integrated genomic analysis that uncovers extensive intra-tumor heterogeneity, with the majority of patients displaying different GB subtypes within the same tumor. Moreover, we reconstructed the phylogeny of the fragments for each patient, identifying copy number alterations in EGFR and CDKN2A/B/p14ARF as early events, and aberrations in PDGFRA and PTEN as later events during cancer progression. We also characterized the clonal organization of each tumor fragment at the single-molecule level, detecting multiple co-existing cell lineages. CONCLUSION: Our results reveal the genome-wide architecture of intra-tumor variability in GB across multiple spatial scales and patient-specific patterns of cancer evolution, with consequences for our understanding of treatment design and therapeutic failure.

## OM-075. PEROXIREDOXIN 1: A PROGNOSTIC MARKER IN ANAPLASTIC GLIOMA WITH PROINVASIVE AND CHEMORESISTANT PROPERTIES

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INTRODUCTION: A genome-wide screen searching for differentially methylated genes in conjunction with $O^6$-methylguanine-DNA methyltransferase (MGMT) promoter methylation status pointed towards a prognostic value of peroxiredoxin 1 (PRDX1) promoter methylation in anaplastic glioma. PRDX1 plays a role in redox signaling and has been ascribed both tumor suppressive and supportive features. In anaplastic gliomas, PRDX1 has been suggested to contribute to the 1p/19q co-deletion phenotype. Here, a role for PRDX1 as a biomarker and potential target for intervention was analyzed. METHODS: PRDX1 methylation status was assessed via MassARRAY in 133 samples from the NOA-04 study. For validation, 80 samples of the German Glioma Network were used. PRDX1 expression levels were examined in glioma cell lines (n = 13) and patient samples (n = 11). Data from The Cancer Genome Atlas (TCGA) were used for statistically relevant correlation of methylation and expression. Analyses for invasiveness and resistance to genotoxic stimuli were performed in Hs683 and T98G glioma cells with a stable lentiviral knock-down of PRDX1. RESULTS: PRDX1 methylation turned out to be an independent prognostic marker for overall and progression-free survival, although methylation of PRDX1 was correlated with isocitrate dehydrogenase 1 (IDH1) mutation and MGMT promoter methylation status. Expression and methylation of PRDX1 were found to be inversely correlated. Hs683 cells with a knock-down of PRDX1 were more clonogenic and within this assay more sensitive to chemotherapy, but not radiotherapy. Furthermore, PRDX1 knock-down cells were less invasive and matrix metalloproteinase 2 (MMP-2) was less active in these cells. CONCLUSION: We determined PRDX1 as an independent prognostic marker in anaplastic glioma. Moreover, PRDX1 is of functional relevance for glioma cell invasiveness and resistance to temozolomide chemotherapy. With that, PRDX1 helps to explain chemosensitivity of anaplastic gliomas with a methylation of PRDX1 beyond the methylator phenotype. Genes mediating radioresistance are still elusive.

## OM-076. DISTINGUISHING RESPONDERS FROM NON-RESPONDERS TO BEVACIZUMAB USING CT PERFUSION

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BACKGROUND: We aimed to determine whether computed tomography (CT) perfusion can distinguish responders from non-responders to Bevacizumab in a pre-clinical model of malignant glioma. METHODS: Rats bearing the C6 glioma were treated with either Bevacizumab (n = 11, "treated", 10 mg/kg every other day for 4 days) or saline (n = 4, "control"). Serial CT perfusion scans were performed before and four times after treatments for a maximum of 10 days. Tumor volume, relative blood flow (rBF), relative blood volume (rBV), permeability-surface area (PS), and mean transit time (MTT) at baseline and at the last scan before sacrifice were compared. RESULTS: Treated subjects with short survival (median 4 days, n = 7) were designated as "non-responders" and treated subjects with longer survival (median >10 days, n = 4) were designated as "responders" (log-rank, $p \leq 0.04$). Interestingly, survival was significantly longer in controls than non-responders (median survival = 8 vs. 4 days; log-rank, $p = 0.04$). All pre-treatment parameters were not different amongst the three groups. Post-treatment tumor volumes were significantly higher in controls than responders ($p = 0.03$). rBF decreased in 3/4 controls ($p = 0.20$) and 6/7 non-responders ($p = 0.10$), while rBF increased in all responders ($p = 0.08$). There were significant differences in percent change in rBF between controls and responders ($p < 0.05$) and between responders and non-responders ($p = 0.01$). Percent change in MTT trended higher in controls than responders ($p = 0.06$), suggesting alleviation of edema in responders. CONCLUSIONS: CT Perfusion studies showed that an increase in rBF after treatment corresponded to improved survival while decreased rBF signaled worse survival compared to controls. Our results suggest that CT perfusion could be an imaging biomarker to select patients who will respond to Bevacizumab.

## OM-77. A SURVEY OF INTRAGENIC BREAKPOINTS IN GBM IDENTIFIES A DISTINCT SUBSET ASSOCIATED WITH POOR SURVIVAL

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With the advent of high-throughput sequencing technologies, much progress has been made in the identification of somatic structural rearrangements

in cancer genomes. However, characterization of the complex alterations and their associated mechanisms remains inadequate. Here, we report a comprehensive analysis of whole genome sequencing and DNA copy number data sets from The Cancer Genome Atlas, to relate chromosomal alterations to imbalances in DNA dosage and to describe the landscape of intragenic breakpoints in glioblastoma multiforme (GBM). Gene length, GC content and local presence of a copy number alteration were closely associated with breakpoint susceptibility. A dense pattern of repeated focal amplifications involving the *MDM2/CDK4* oncogenes and associated with poor survival was identified in 5% of GBMs. Gene fusions and rearrangements were detected concomitant within the breakpoint enriched region. At the gene level, we noted recurrent breakpoints in genes such as apoptosis regulator *FAF1*. Structural alterations of *FAF1* gene disrupted expression and led to protein depletion. Restoration of FAF1 protein in glioma cell lines significantly increased the FAS mediated apoptosis response. Our study uncovered a previously underappreciated genomic mechanism of gene deregulation that can confer growth advantages on tumor cells and may generate cancer-specific vulnerabilities in subsets of GBM. [Genes. Dev. 2013 Jul 1;27(13):1462-72.]