primary vs. secondary patients. Preliminary results of single-gene analysis of MC tumours showed FOXM1, MYBL2, TOP2A, BIRC5 expression was higher in WHO grade III samples. Gene-expression signatures in the individual patients and gene ontology enrichment analyses are in process. CONCLUSIONS: FOXM1, MYBL2, TOP2A, BIRC5 RNA expression levels seem to rise during malignant progression across patients. Gene-expression analysis using the Nanostring technology is feasible and a potentially powerful tool to distinguish meningiomas prone to malignant transformation from truly benign meningiomas.

### OTEH-4. DEEPER INSIGHT INTO INTRATUMORAL HETEROGENEITY BY MRI AND PET-GUIDED STEREOTACTIC BIOPSIES FROM GLIOBLASTOMA PATIENTS

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Glioblastoma is one of the most aggressive cancers, but the molecular evolution is still not fully understood. We used PET imaging combined with deep sequencing of glioblastoma biopsies at both the RNA and DNA levels to get a deeper insight into molecular evolution. In the clinical setting, PET imaging provides information about metabolically active tumor areas, but the molecular interpretation is unclear. Our primary objective was to perform an intratumoral spatial comparison of biopsies from potentially aggressive and less aggressive areas in glioblastomas according to PET scans. Additionally, tissue from the tumor periphery was included. We used MRI, $^{11}$C-methionine(MET) PET, and $^{18}$F-FDG PET was used in combination to obtain a series of neurosurgical stereotactic biopsies from tumor areas with high MET and $^{18}$F-FDG uptake (hotspot), low MET and $^{18}$F-FDG uptake (coldspot), as well as tumor periphery of six glioblastoma patients that were processed for whole genome, exome, and transcriptome sequencing. Differential gene expression and gene ontology analysis showed that hotspots were enriched in gene sets associated with DNA replication, cell cycle, and ligand receptor interaction. Genome and exome analysis suggested hotspots and coldspots to have similar mutational profiles. However, a limited number of hotspot-specific mutations and fusion transcripts indicated that hotspot tumor cells developed from coldspot cells and point at the potential role of hotspot driver genes in glioblastoma. Our findings reveal that hotspots in glioblastomas represent a more advanced stage of molecular evolution than coldspots.

### OTEH-5. CHARACTERIZATION OF LONG-NON CODING RNA ASSOCIATED CERNA NETWORK HUB GENE INVOLVED IN GLIOBLASTOMA MULTIFORME LIPID METABOLISM

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BACKGROUND: Glioblastoma multiforme (GBM) are the major death contributor in primary brain tumour. Despite having an improved diagnostic criterion by integrating both histological and molecular features such as Isocitrate Dehydrogenase (IDH) detection, the prognosis of GBM patients still remain poor. Lipid metabolism is an essential pathway that fuel GBM aggressiveness. IDH1 one of the key enzyme that regulates it. Long non-coding RNAs (lncRNAs) act as competing endogenous RNAs (ceRNAs) in tumour initiation and progression. In parallel, miRNA-mediate ceRNA crosstalk between lncRNAs and mRNAs. In this study, we aim to investigate the IDH1 subgroup lncRNA associated ceRNA network hub gene responsible in the coordination of glioblastoma multiforme lipid metabolism using bioinformatics approach. METHODS: TCGA-GBM dataset consist of 168 GBM RNA-seq (159 IDH1 wt and 9 IDH1 mutation) were downloaded. Differentially expressed genes (DEG) were then obtained using Limma. Gene sets related with lipid metabolism from GSEA-MSigDB were overlapped with DEG using Venn diagram to identify the DEmRNA that are related with lipid metabolism. Construction of mRNA-miRNA and lncRNA-miRNA interaction networks were performed using miRNet. The ceRNA interaction network were later combined in the Cytoscape software. Potential lncRNA hub genes were identified by CytoHubba analysis. RESULTS: From 1389 DEG, 67 genes were identified to be significant in the regulation of lipid metabolism. By analysing the lncRNA-miRNA-mRNA interaction network, candidate hub lncRNAs consists of three genes with highest connective nodes; CYTOR, LOXL1-AS1 and HOTAIR. These genes are significantly upregulated in glioma. LOXL1-AS1 serve as an excellent prognostic biomarker for both glioma and glioblastoma as the effect of high and low LOXL1-AS1 expression on patients' survival is significant ($p<0.05$). CONCLUSIONS: Data mining and bioinformatics approach guided the identification of the potential hub lncRNAs associated ceRNA network in GBM lipid metabolism. This allows us to uncover the novel role of lncRNA in GBM tumorigenesis.

### OTEH-6. ALGORITHMIC APPROACH TO CHARACTERIZE POST-TREATMENT RECURRENT GLIOMA USING RNA SEQUENCING AND QUANTITATIVE HISTOPATHOLOGY

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INTRODUCTION: Distinguishing between tumor and treatment effect in post-treatment glioma, although crucial for clinical management, is difficult because contrast-enhancing regions are mixtures of recurrent tumor and reactive tissue, and definitive histopathological criteria do not exist. This study disentangles the marked intra-tumoral heterogeneity in the treatment-recurrent setting by developing an unsupervised framework to algorithmically categorize intraoperative MRI-localized biopsies into three clinically-relevant tissue clusters based on joint analysis of RNA sequencing and histopathological data. METHODS: A retrospective cohort of 84 MRI-localized biopsies from 37 patients with post-treatment recurrent glioblastoma underwent mRNA extraction and quantification via PLATEseq protocol. For 48 of 84 biopsies, a neighboring piece of tissue underwent quantitative histopathology based on labeling index (LI) for SOX2, CD68, NeuN, Ki67, and H&E. Correlation between LIs and gene expression for these 48 samples was performed. Genes significantly correlated ($p<0.05$) with ≥1 marker were used for hierarchical clustering of correlation matrix, identifying three mutually-exclusive tissue-specific gene sets. These sets were then used to perform ssGSEA to categorize each of 84 biopsies into one of three tissue types. RESULTS: Correlation analysis identified 7779 genes significantly correlated with ≥1 histopathological marker. Clustering revealed three gene sets associated with specific markers: SetA-3688 genes associated with SOX2/Ki67/H&E; SetB-2418 genes associated with CD68; SetC-1673 genes associated with NeuN. ssGSEA using these sets categorized each biopsy into one of three tissue types: 27 biopsies enriched in SetA, 28 in SetB, and 29 in SetC. CONCLUSIONS: Using MRI-localized biopsies with both RNAseq and histopathological data, this algorithmic approach allows development of three orthogonal tissue-specific gene sets that can be applied to characterize the heterogeneity in post-treatment recurrent glioma: SetA: genes correlated with SOX2/Ki67/H&E, representing recurrent tumor; SetB: genes correlated with CD68 (microglia) representing reactive tissue consistent with treatment effect; SetC: genes correlated with NeuN (neurons), representing infiltrated brain.

### OTEH-7. MOLECULAR CHARACTERIZATION OF TUMOR STIFFNESS IN GLIOBLASTOMA

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Tumor heterogeneity is one of the hallmarks of glioblastoma multiforme (GBM). Morphology within a given GBM tumor can be extremely variable where some regions of the tumor have a soft, gel-like structure while other areas are dense and fibrous. Abnormal mechanical stress and tissue stiffening caused by cancer proliferation are believed to affect vascularity by compressing structurally weak blood vessels and restricting the supply of nutrients and oxygen to the tissue. These effects contribute to a hypoxic microenvironment that promotes disease progression and chemoresistance. The genetic and molecular mechanisms that govern tissue stiffness within GBM tumors, however, are largely unknown. Magnetic Resonance Elastography (MRE) is an emerging technique for quantifying tissue stiffness non-invasively. We have evaluated 10 GBM patients by MRE imaging obtained prior to surgical resection. During surgery, 2–7 stereotactically navigated biopsies were collected from locations within the tumor with varying degrees of measured stiffness. Biopsies were processed to extract RNA, proteins, polar metabolites and lipids. Biomolecules were analyzed on relevant -omics platforms (RNA sequencing, MS-proteomics and lipidomics, NMR of polar metabolites). Differential expression and gene set enrichment analysis of patient paired biopsies indicate an overall increase in macrophage infiltration and extracellular matrix re-organization associated with increased tumor stiffness. Among the most highly upregulated genes in stiff tumor tissue were lymphatic endothelial hyaluronic acid receptor 1 (LYVE-1) and macrophage receptor with collagenous structure (MARCO), both of which have been associated with immune cell infiltration and tissue stiffness. Our preliminary findings offer novel insights into tumor morphology in GBM that can be inferred from imaging prior to surgery. This can be used to identify tumor regions with high risk of progression and infiltration, thereby informing and guiding surgical strategy and may ultimately lead to novel treatment strategies.

### OTEH-8. PATHWAY-BASED APPROACH REVEALS SENSITIVITY TO RADIATION WHEN TARGETING E2F1 IN GLIOBLASTOMA

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The great phenotypic heterogeneity of glioblastoma (GBM) – both inter and intratumorally – has hindered therapeutic efforts. While genome-based molecular subtyping has revealed that GBMs may be parsed into several molecularly distinct categories, this insight has not translated to a significant extension of patient survival. We hypothesize that, rather than gene expression as a whole, analysis of targetable pathways could yield important insights into the development of novel classification schemes and, most importantly, to targeted therapeutics. Here, we interrogated tumor samples using a pathway-based approach to resolve tumoral heterogeneity. The Cancer Genome Atlas samples were clustered using gene set enrichment analysis and the resulting 3 clusters were informative of patient survival and only modestly overlapped with prior molecular classification. We validated our approach by generating gene lists from common elements found in the top contributing genesets for a particular cluster and testing the top targets in appropriate gliomasphere patient-derived lines. Samples enriched for cell cycle related genesets showed a decrease in sphere formation capacity, proliferation and in vivo tumor growth when E2F1, our top target, was silenced. Consistent with our theory, E2F1 knockdown had little or no effect on the growth of the non-enriched lines, despite their ability to proliferate in vitro and in vivo. We similarly analyzed single cell RNAseq datasets and correlated cell cycle and stemness signatures with the gene lists we generated, concluding that cells with stem cell signatures were depleted of E2F1 and its downstream targets. Finally, we confirmed a connection between E2F1 and cellular inhibitor of PP2A (CIP2A) in a cluster of samples. Loss of function studies reveal a diminished capacity for DNA damage regulation in E2F1 activated samples. Our studies relate inter- and intratumoral heterogeneity to critical cellular pathways dysregulated in GBM, with the ultimate goal of establishing a pipeline for patient- and tumor-specific precision medicine.

### OTEH-9. SCRNA SEQUENCING OF PRONEURAL GBM AVATAR MODEL REVEALS ACQUISITION OF ONCOGENIC TRANSCRIPTIONAL PROGRAMMING AND INFERS A DEVELOPMENTAL PATH TOWARDS A GENOMICALLY UNSTABLE STATE

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Glioblastomas (GBMs) are the most common malignant primary brain tumors, and the paucity of novel treatments warrants an investigation of its origins and development into aggressive, lethal tumors. Koga & Chaim *et al.* have recently shown that human pluripotent stem cells (hiPSCs) with different combinations of driver mutations can be differentiated into neural progenitor cells (NPCs) and engrafted into mice to form high grade gliomas (iHGGs). In this work, scRNA seq analysis was used to investigate the development of *TP53−/−;PDGFRAΔ8–9* iHGGs, an avatar model that has been shown to recapitulate the proneural subtype of GBM. After re-engrafting the primary avatar cultures (secondary tumor stage), the *TP53−/−;PDGFRAΔ8–9* iHGGs developed diverse transcriptional programming and acquired a subpopulation of cells with high expression of known GBM oncogenes, such as MYC, CDK4, and PDGFRA. Notably, when all datasets were aggregated, this oncogene amplifying transcriptional program became the largest source of variation between all stages and replicates of the *TP53−/−;PDGFRAΔ8–9* iHGGs. Indicated by a larger total copy number variation (CNV), this oncogene-amplifying program was associated with a genomically unstable developmental state. Trajectory inference could track the development of this population from the initial primary culture of *TP53−/−;PDGFRAΔ8–9* iHGG. Differential gene expression analysis identified distinct divergences in clonal evolution—e.g., high expression of the S100 protein family in one cluster—following the acquisition of this genomically unstable state. Lastly, genomic PCR was used to ascertain whether these changes in transcriptional programming were reflected in changes in DNA copy number and identified DNA amplifications of MYC and CDK4. Our scRNA seq analysis of the GBM avatar model platform provides novel insight into how oncogenic states in GBM develop from a small number of driver mutations.

### OTEH-10. EVOLUTIONARY TRAJECTORY OF EPIGENOMIC OF GLIOMAS

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Gliomas are the most common malignant brain tumor, have an aggressive behavior, and invariably relapse and progress. Despite the recent advancements, little is known about the role of the epigenome in glioma disease progression and recurrence. To investigate the molecular dynamics over time and in response to therapeutic pressures, the Glioma Longitudinal AnalySiS (GLASS) Consortium, a multinational collaboration, is investigating epigenome-wide molecular data from primary and recurrent matched pairs, including IDH mutant (IDHmut) and IDH wildtype (IDHwt) gliomas. We have compiled a total of 357 samples comprising 143 primary-recurrent pairs profiled by DNA methylation, of which 157 samples have genomic data (WXS/WGS) and 120 have transcriptomic data (RNAseq). IDHwt gliomas have a distinct epigenetic evolution compared to IDHmut after treatment. IDHwt gliomas are more epigenetically stable over time, while IDHmut gliomas display a loss of DNA methylation throughout disease progression. Next, we investigated the molecular drivers of longitudinal gliomas by integration of DNA methylation and gene expression data. We identified epigenetic activation of cell cycle pathways in recurrent IDHmut compared to initial tumors. Transcription factors musculin, ZNF367, and ZNF682 are enriched among recurrent IDHmut gliomas and potentially regulate IDHmut recurrence and/or progression. We next used a DNA methylation-based deconvolution approach to estimate the tumor microenvironment (TME) composition. We found that the TME among IDHmut subtypes (Codel, GCIMP-high, and GCIMP-low) presented less immune infiltration than IDHwt (Classic-like, Mesenchymal-like, and PA-like). Post-treatment, we found a decrease of CD4+T and an increase of CD8+T cells in IDHmut. In conclusion, IDHmut gliomas present a more unstable epigenome, while the epigenome of IDHwt gliomas seems relatively preserved after treatment. We identified potential master regulators of cell cycle deregulation of IDHmut recurrence. Finally, the TME differs across IDHmut and IDHwt gliomas and the cell composition changes over time.

### OTEH-11. SINGLE CELL RNA SEQUENCING TO IDENTIFY CELLULAR HETEROGENEITY WITH IN PITUITARY ADENOMAS

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Pituitary adenomas (PA) are one of the most common primary brain tumors and comprise approximately 15% of brain neoplasms. Most PA are