increased frequency of CNAs. Longitudinal expression analysis showed changes in expression of >800 genes, including an up-regulated locus enriched with histone genes. Further integrative analyses are ongoing and will be reported at the meeting.

OS08.2.A. THREE LAYERS OF NEURONAL-LIKE MECHANISMS DRIVING BRAIN TUMOR METASTASIS
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BACKGROUND: Glioblastoma are characterized by their infiltration into the whole brain. Membrane protrusions of glioma cells called tumor microtubes are subcellular structures contributing to glioma cell invasion. They are the anatomical building block to build a functional and therapy-resistant tumor cell network interconnected by tumor microtubes (TMs) that characterizes a glioblastoma cell subpopulation, while other subpopulations appear unconnected to other glioma cells. The biological role of glioblastoma cells lacking connections with each other remains unclear. Furthermore, how neuroglial interactions influence this subpopulation is unclear. MATERIAL AND METHODS: Time-lapse in vivo two-photon microscopy and single-cell RNA-sequencing are combined to characterize functional different subpopulations of glioblastoma cells. Intravital, augmented microscopy, three-dimensional calcium imaging, electrophysiology and volume electron microscopy are used to investigate the role of neuronal invasion mechanisms and the role of neuroglial synapses for tumor microtube generation and dynamics. RESULTS: In vivo imaging revealed that glioblastoma cells lacking connections to other tumor cells and astrocytes were the main subpopulation driving glioblastoma invasion. These cells were characterised with single-cell RNA-sequencing. This revealed that this subpopulation is enriched for neuronal, neural progenitor-like, and non-neuronal-like cell states as previously described. Sparsely enriched with tumor-cell and astrocyte-unconnected neurons, these cells evolve over time into regions with tumor-cell and astrocyte-connected, glioblastoma cell networks reflected by molecular cell state changes that are reflected in cell state changes in different regions of human glioblastoma. In conclusion, mechanisms of glioblastoma cell cell migration resemble neuronal and neural progenitor patterns during brain development. Lastly, neuronal activity stimulated neuroglial synapses and subsequently increased glioblastoma cell invasiveness by stimulating generation of new tumor microtubes and enhanced tumor microtube dynamics. CONCLUSION: This study uncovers three novel layers of neuronal features driving glioblastoma cell invasion. We are able to connect molecular, cellular heterogeneity and functional glioblastoma cell states interlinking heterogeneity and dissemination of glioblastoma, two important hallmarks of this disease. Lastly, this study delineates a potential roadmap to clinical translation with the multidimensional characterisation of human glioblastoma.

OS08.3.A. DISTINCT SYSTEMIC AND TUMOR MICROENVIRONMENT IMMUNE LANDSCAPES DISCRIMINATE ACROSS SELLAR TUMOR TYPES AND CONTROLS THROUGH A METHYLATION-BASED DECONVOLUTION METHOD

BACKGROUND: Systemic (Sys) and tumor microenvironment (TME) immune milieux play a pivotal role in tumor development, outcome and immunotherapy response predictions across a variety of central nervous system tumors. Genome-wide methylation profiling can reliably discriminate and estimate immune cell components present in the blood and within the tumor and has not been reported across sellar tumor subtypes (STT). MATERIAL AND METHODS: We estimated cell composition in liquid biopsy (LB, serum/plasma) and tissue specimens from 42 STT collections (i.e., pituitary neuroendocrine tumours [PitNETs; n=37] and craniohypophyseomas [CP; n=5]), and 26 non-tumor controls (LB: 11; Tissue: 15) using MethylCIBERSORT, a methylation-based deconvolution algorithm and established immune cell signatures as reference. LB composition was proxied with WBC absolute counts. Immune cell proportions across sample sources were explored (Spearman). Immune cell proportion hierarchical k-means clustering was performed across tissue and LB specimens. Similarly, mean comparisons between and across sample types and level of interest were performed.RESULTS: We identified three immune-clusters across tissue specimens which distinguished controls (k3-cluster) from sellar tumor subtypes (k1- and k2-clusters), primarily attributable to different monocyte subpopulations. Immunocytochemistry analyses on tissue and CP belonging to the k2-cluster, presented a distinct immune profile compared to their k1-sellar tumor counterparts. Analysis of plasma-derived immune clusters revealed unconnected to other glioma cells. The biological role of glioblastoma cells lacking connections with each other remains unclear. Furthermore, how neuroglial interactions influence this subpopulation is unclear. MATERIAL AND METHODS: Time-lapse in vivo two-photon microscopy and single-cell RNA-sequencing are combined to characterize functional different subpopulations of glioblastoma cells. Intravital, augmented microscopy, three-dimensional calcium imaging, electrophysiology and volume electron microscopy are used to investigate the role of neuronal invasion mechanisms and the role of neuroglial synapses for tumor microtube generation and dynamics. RESULTS: In vivo imaging revealed that glioblastoma cells lacking connections to other tumor cells and astrocytes were the main subpopulation driving glioblastoma invasion. These cells were characterised with single-cell RNA-sequencing. This revealed that this subpopulation is enriched for neuronal, neural progenitor-like, and non-neuronal-like cell states as previously described. Sparsely enriched with tumor-cell and astrocyte-unconnected neurons, these cells evolve over time into regions with tumor-cell and astrocyte-connected, glioblastoma cell networks reflected by molecular cell state changes that are reflected in cell state changes in different regions of human glioblastoma. In conclusion, mechanisms of glioblastoma cell cell migration resemble neuronal and neural progenitor patterns during brain development. Lastly, neuronal activity stimulated neuroglial synapses and subsequently increased glioblastoma cell invasiveness by stimulating generation of new tumor microtubes and enhanced tumor microtube dynamics. CONCLUSION: This study uncovers three novel layers of neuronal features driving glioblastoma cell invasion. We are able to connect molecular, cellular heterogeneity and functional glioblastoma cell states interlinking heterogeneity and dissemination of glioblastoma, two important hallmarks of this disease. Lastly, this study delineates a potential roadmap to clinical translation with the multidimensional characterisation of human glioblastoma.

OS08 HIGHLIGHTS FROM PRECLINICAL ABSTRACTS

OS08.1.A. INTEGRATIVE MOLECULAR ANALYSIS OF MATCHED PRIMARY AND RECURRENT IDH-MUTANT ASTROCYTOMA: AN UPDATE FROM THE GLASS-NL CONSORTIUM

BACKGROUND: The evolutionary processes that drive tumor progression in patients with IDH-mutant astrocytoma remain largely unclear. The GLASS-NL consortium was initiated to gain insight into the molecular mechanisms underlying glioma evolution and to identify markers of progression and aggressive glioma subtypes. Ultimately, such markers can assist clinical decision making. Here, we present the DNA methylation profiling, RNA-sequencing and shallow whole-genome sequencing (sWGS) of samples included in the GLASS-NL study. METHODS: Eligible were patients with an IDH-mutant, 1p19q non-codeleted, astrocytoma at first diagnosis and who underwent surgical resection of the tumor at least twice separated by >6 months, and of whom paired tumor samples were available for analyses. Overall survival (OS) was measured from date of initial surgery. DNA methylation profiling was performed with the 850kEPIC array, and transcriptome and sWGS data by NGS. After quality control, DNA methylation data of 103, expression data of 91, and sWGS data of 92 patients was available for further analysis. Methylation classes were determined according to Varambally et al. and copy number alterations (CNAs) were extracted from both sWGS and DNA-methylation data. RESULTS: 110 patients were identified from various medical centers in the Netherlands. The median time between surgical resections was 41.9 months (IQR:26.5-65.9) and after initial surgery, 63% and 22% of the patients were treated with radiotherapy or chemotherapy respectively. The proportion of samples assigned to the high grade methylation class increased -three-fold at recurrence. 85% of patients that progressed from low to high grade, received treatment prior to recurrence compared to 53% of the patients that remained low grade.

With sWGS and DNA methylation analyses of paired samples, we identified CNAs that were more frequently altered in high grade samples. Univariate analysis showed that losses in 3p, 1p, 1q, 14q and 14q were associated with poor OS. More than 800 differentially expressed genes between initial and recurrent tumor samples were found. Chromosome enrichment analysis revealed a locus on chromosome 6 enriched with histone genes, to be significantly up-regulated over time. CONCLUSION: Longitudinal methylation profiling of IDH-mutant astrocytoma reveals a shift towards a higher grade at tumor recurrence coinciding with reduced DNA methylation levels, and
enrichment of CD4+(including the regulatory subtype), CD8 and CD56-T and depletion of natural killer cells. Differences across serum- and tissue-derived clusters were present but less prominent than their plasma counterparts. No correlation was observed between immune cell proportions and clinical features within each tumor type (sex, age, histotypes, invasion etc.) was observed.

CONCLUSION: Our results suggest that protein levels are characterized by differential TME and systemic immune subtypes which also distinguish these tumor microenvironments. Additionally, differences in the compartmentalization between tissue and LB sources, more readily observed in plasma, suggest that the systemic response to the presence of the tumor is distinct from the immune response noted in the TME. Tumor immune subtyping may allow the stratification of patients according to immunotherapy response vulnerabilities.

OS08.4.A. ANALYSIS OF MELANOMA BRAIN METASTASIS IMMUNE MICROENVIRONMENT THROUGH MULTIPLE GENETIC PROFILING

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1Background: Novel immunotherapies based on targeting of specific immunomodulatory pathways have shown great promise in the treatment of metastatic melanoma, but melanoma brain metastases (BM) remain an unmet oncological need with an overall 2-year survival rate lower than 10%. Tumour immune microenvironment has been demonstrated to play a key role in BM establishment and development, but data regarding the specific milieu of tumour immune microenvironment (TME) is limited.

MATERIAL AND METHODS: Gene expression profiles of 55 samples of primary melanoma and BM were evaluated using the nCounter PanCancer IO 360 Panel (NanoString Technologies) targeting >770 mRNA involved in tumour immune microenvironment modulation. TME profiles in primary melanomas and their 10 matched BM, 23 unmatched BM, and 10 locally advanced control melanomas without evidence of BM after >5 year follow up.

RESULTS: Among BM samples, most patients (25/45) were males and median age at BM diagnosis was 61.2 years with a median time to BM development of 21.7 years. Median OS from BM diagnosis was 1.3 years. Several genes resulted significantly downregulated in BM compared to primary melanomas, including SERPINB3 (p<0.001), ARHG (p<0.0067), S100A8 (p<0.001), S100A9 (p<0.001), S100A12 (p<0.0037), IL1RN (p=0.0012), CCL2 (p=0.0012), CCL2 (p=0.0012) and CCL13 (p=0.037); conversely, C7 was upregulated (p<0.001). Downregulated signatures in BM involved those associated with multiple immune cell populations, including neutrophils, dendritic cells, mast cells and Treg, as well as inflammatory chemokines, the CTLA4 immune checkpoint and ARF1 enzyme function; conversely, MAGE-A-related signature was upregulated. Comparison between primary melanomas which developed BM and those which did not show a significant overexpression of RRM2 (p=0.0247) and TNFRSF1A (p=0.032) genes in the latter group and an upregulation of the PI3K-P1 pathway. Analysis according to tumor mutational status showed an upregulation of signatures associated with inflammatory chemokines, dendritic and myeloid cells and neutrophils. No differences were observed according to time to BM development and survival from BM diagnosis.

CONCLUSION: Our findings show that melanoma BM harbor distinct immunosuppressive mechanisms compared to primary tumors: this data elucidates the importance of investigating the heterogeneity of BM microenvironment. Genes and pathways selectively overexpressed or downregulated in melanoma BM should be validated to be possibly considered as novel therapeutic targets.

OS08.5.A. ADENOVIRUS-MEDIATED DELIVERY OF THE MHC-II IMMUNOMODULATORY GENE CIITA TO MALIGNANT GLIOBLASTOMA TUMOR CELLS: KILLING IN IMMUNOCOMPETENT Glioblastoma ORGANOGOSIS

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Background: Although immunotherapies represent an encouraging alternative to current, to date non-effective treatments in Glioblastoma (GBM). One aspect contributing to this failure is the highly immunosuppressive GBM microenvironment. Our approach to overcome immunosuppression is to increase anti-tumor immune responses via adenovirus (AdV)-mediated delivery of the MHC-II Transactivator (CIITA) gene. CIITA-induced MHC-II expression is anticipated to convert GBM cells into surrogate antigen presenting cells able to prime T helper cells, therefore promoting CD4+ and CD8+ mediated immunity. MATERIAL AND METHODS: We generated AdVs containing wild type CIITA (Ad-CIITA) using a replication-defective serotype5 adenoviral backbone. AdVs containing a mutated, non-functional version of CIITA (Ad-CIITA mutant) and an empty CMV promoter (Ad-null) were used as controls. AdV-mediated MHC-II expression was monitored at mRNA, protein and cell surface level. For the functional assessment of anti-tumor immune responses, we developed an advanced human GBM organoid model of gliomas with immunocompetent co-culture, either human peripheral blood mononuclear cells (PBMCs) or isolated CD3+ T cells. T cell mediated tumor cell killing was monitored over time via live cell imaging and flow cytometry. RESULTS: We successfully constructed and produced a CIITA-armed AdV that induces MHC-II expression in infected GBM cells, indicating the efficient expression of transgene in active CIITA for at least 24h post infection. In immunocompetent human GBM organoids, Ad-CIITA infection of tumor cells led to prominent organoid disruption and tumor cell death, an effect that was not observed in the absence of PBMCs or CD3+ T cells. Tumor organoids infected with Ad-CIITA or Ad-CIITA mutant harbored distinct implication of cell surface MHC-II molecules in the observed phenotype. CONCLUSION: Our results demonstrate that AdV-mediated delivery of CIITA is a promising strategy to increase T cell mediated immunity against glioblastoma.

OS08.6.A. NOVEL ROLE OF ARF4-MEDIATED RETROGRADE TRANSPORTING AS A DRIVER OF CHEMORESISTANCE IN GBM

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Background: Glioblastoma (GBM) is the most common type of adult malignant brain tumor, with a median survival of only 21 months. This is primarily attributed to the high rate of recurrence and resistance to standard therapies. The transcription factor ARF4 has been implicated in the development and plasticity of glioblastoma, and thus may play a role in the development and maintenance of chemoresistance. A recent study found that ARF4 knockdown in PDX MBM-314 increased TMZ sensitivity. However, the mechanism underlying this increase in TMZ sensitivity is unknown.

Methodology: We generated a TMZ-resistant and ARF4-overexpression cell line, for which we used an unbiased proteomics screen to identify which genes were upregulated in TMZ-resistant conditions and elevated in ARF4-overexpression and TMZ-treated conditions. We then performed an unbiased proteomics screen to identify which genes were significantly heightened sensitivity to TMZ in multiple GBM patient-derived organoids and a GBM cell line.

Results: We identified ~200 novel genes, including a previously unstudied transporter, CRISPR-Cas9 sensitivity screen as well as a variety of in vitro and in vivo experiments, such as live-cell imaging, cell viability assays, and western blotting were conducted. RESULTS: Initial investigation into ARF4 showed significant elevations in expression at RNA and protein levels (p<0.05) in patient recurrent tumors, as well as a significant survival benefit in patient datasets when downregulated (p<0.05). Knocking out ARF4 resulted in significantly heightened sensitivity to TMZ in multiple GBM patient-derived xenograft lines and extended survival compared to the controls (p<0.01) in vivo. Further investigation revealed that ARF4 overexpression resulted in a retrograde transport mutant, revealed that ARF4 knockdown significantly inhibited retrograde trafficking, while ARF4 overexpression resulted in an upregulated increase in retrograde trafficking in vitro. This effect was also seen in ARF4 knockdown and CIITA expressing cells, which displayed enhanced trafficking dynamics, suggesting that ARF4-mediated retrograde trafficking is elevated during therapy to drive nuclear localization of key chemoresistance-promoting factors. We then performed an unbiased proteomics screen to identify which genes were being uniquely transported to the nucleus as a product of ARF4-mediated retrograde trafficking, which revealed enrichment of the EGFR signaling pathway in particular. Validation experiments confirmed a decrease in EGFR trafficking and nuclear EGFR expression in ARF4-knockdowns and an increase in EGFR trafficking and nuclear EGFR expression in ARF4-overexpression and TMZ-treated conditions. Furthermore, nuclear DNA-PK, a DNA repair protein known to be transcriptionally activated by EGFR, was similarly found to be downregulated in ARF4-knockdown conditions and elevated in ARF4-overexpression and TMZ-treated conditions.

Conclusion: Here, we show that ARF4 may be responsible for promoting chemoresistance through altered retrograde trafficking of EGFR specifically. Thus, our study has yielded a promising and novel therapeutic target for GBM, a disease desperately in need of new therapeutic strategies.

OS08.7.A. LUMOSTINE AND THE IMMUNOCYTOKINE 1-91TFE ARE A PROMISING TREATMENT COMBINATION FOR RECURRENT Glioblastoma

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Background: Treatment options for recurrent glioblastoma are limited and with the possible exception of regorafenib, no agent has demonstrated superior activity to lomustine. Therefore, there is an urgent need for more effective treatment strategies for recurrent glioblastoma. Here, we investigated different treatment combinations based on the tumor-stroma targeting of a lymphokine.