Abstracts

Cellular components of the immune system. While immunotherapies have recently revolutionized cancer therapy, they have shown so far little therapeutic success in glioblastoma patients. To enhance the efficacy of novel strategies, we need to better understand the immunogenic status of glioblastoma cells and their cross-talk with immune cells in different microenvironmental niches. MATERIAL AND METHODS: We assessed expression of molecules related to antigen processing and presentation and activation markers on microglial cells using antibodies against a panel of glioblastoma patient-derived organoids, 3D stem-like cultures and adherent cell lines under varying microenvironmental conditions (varying oxygen levels, inflammation). We further established an allogenic co-culture protocol for glioblastoma organoids with immune cells isolated from HC-sourced donor blood, allowing for the functional assessment of the crosstalk between tumor and immune cells. RESULTS: Analysis of a large cohort of patient tumors and patient-derived glioblastoma preclinical models shows inter-patient heterogeneity at the level of components of microenvironment which includes complex (MHC-I complexes, Gata3 checkpoints, Glioblastoma cells in general express MHC-I machinery, albeit at different levels. MHC-II and immune checkpoints are variably expressed across glioblastoma cells. Different tumor microenvironment conditions, including hypoxia and interferon-γ, impact the expression of immune-related molecules. Upon co-culture, HLA-matched donor-derived T cells integrate well into the core of glioblastoma tumor organoids and display reciprocal cross-talk with tumor cells. CONCLUSION: Assessing antigen presentation and immune cell recruitment at the functional and transcriptional level are key to improve specific responses to immunotherapies. Advanced glioblastoma organoids incorporating the immune compartment appear as clinically-relevant models for ex vivo efficacy studies.

P12.14.A. THE ROLE OF ONCO-METABOLITE (R2HYDOXYGLUTARATE) IN THE IDH MUTANT GLIOBLASTOMA MICROENVIRONMENT

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BACKGROUND: Recently, we found that the reactive astrocytes in the IDH wt tumors contribute to tumor immunity and support oncogenic signaling. Here, we characterized the transcriptomic signature of IDH1/2-mutant glioma associated astrocytes and determined a unique inflammatory transformation, profoundly different to astrocytes in IDH wt glioma and due to an onco-metabolite R-2-hydroxyglutarate using Next generation sequencing and human ex-vivo slice model. MATERIAL AND METHODS: We purified and transcriptionally profiled astrocytes from 9 patients with confirmed IDH-R132H mutation, by means of RNA-sequencing and the data were analyzed using the established pipelines. We also used spatial transcriptomics to show how the astrocytes of IDH mutated/wildtype glioma samples. We validated our findings using human organotypic slice model inoculated with IDH-mutant cell line or treated with oncometabolite 2-hydroxyglutarate. Additionally, LC-MS was further used to give us a chart of neurotransmitters due to altered microenvironment. RESULTS: Our results from RNA sequencing showed a transcriptional transformation of reactive astrocytes within the microenvironment of IDH-mutated tumors compared to wtgliaoma by means of RNA-sequencing of purified astrocytes. And by our established human microglia/astrocyte co-culture model inoculated with IDH mutant tumor and R-2HG treatment, we showed that we were able to activate inflammatory transcriptional programs in astrocytes, mediated by the presence of microglia. Further, by spatially mapping the transcriptomic profiles of purified microglia, we were able to confirm that microglia also demonstrate inflammatory activation in IDH mutated glioma. This inflammatory astrocyte transformation is associated with a loss of neurotransmitter homeostasis (disrupted levels of glutamate) in the treated sections, as has been previously reported in IDH mutated tumors. Additionally, R-2HG increased neuronal spiking rate in, pointing to potential excitotoxicity. CONCLUSION: Our work provides a crucial contribution towards understanding the role of R-2HG in the IDH mutant glioma microenvironment and sheds light on the significant microenvironmental differences to IDH wt-glioma.

P12.15.B. ASTROCYTE IMMUNOMETABOLIC REGULATION OF THE GLIOBLASTOMA MICROENVIRONMENT DRIVES TUMOR PATHOGENICITY

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BACKGROUND: Malignant brain tumors are the cause of a disproportionate level of morbidity and mortality among cancer patients, an unfortunately statistic that has remained constant for decades. Despite considerable advances in the molecular characterization of these tumors, targeting the cancer cells has yet to produce significant advances in treatment. An alternative strategy is to target cells in the glioblastoma microenvironment, such as tumor associated astrocytes. Astrocytes control multiple processes in health and disease, ranging from maintaining the brain’s metabolic homeostasis, to modulating neuroinflammation. However, their role in glioblastoma pathogenicity is not well understood. MATERIAL AND METHODS: Immuno-competent mice were implanted with murine glioma cell lines and the role of the tumor associated astrocytes in the tumor pathogenicity was analyzed, and further investigating using in-vitro cultures. RESULTS: Here we report that depletion of reactive astrocytes regresses glioblastoma and prolongs mouse survival. Analysis of the tumor-associated astrocyte transcriptome, revealed that astrocytes initiate transcriptional programs that shape the immune and metabolic compartments in the glioma microenvironment. Specifically, their expression of IFNgamma, CCL2 and CSF1 governs the recruitment of tumor-associated macrophages and promotes a pro-tumorigenic macrophage phenotype. Concomitantly, we demonstrate that astrocyte-derived cholesterol is key to glioma cell survival, and that targeting astrocyte cholesterol efflux, via ABCA1, halts tumor progression. In summary, astrocytes control glioblastoma pathogenicity by re-programming the immunological properties of the tumor microenvironment and supporting the non-oncogenic metabolic dependency of glioblastoma on cholesterol. CONCLUSION: These findings suggest that targeting astrocyte immunometabolic signaling may help treat this uniformly lethal brain tumor.

P13.01.A. CHALLENGES AND SOLUTIONS FOR ESTABLISHING A CNS TUMOR REGISTRY IN AFRICA

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BACKGROUND: CNS tumor registries (CTR) has evolved to a key tool for data collection, evaluation of diagnostic and treatment of patients suffering from tumors of the central nervous system (CNS) in the US, but CTR in Africa does not yet exist. In comparison to high-income countries (HIC), many low- and middle-income countries (LMC) do not yet have national or central CNS tumor registries. Furthermore, appropriate diagnostic steps like MRI and pathological analysis are still scarce in many LMIC. Improving the availability of CTR in resource- limited regions could allow better understanding of some specificities of CNS including incidence, prevalence, mortality and morbidity. However, CTR, MRI and pathological analysis tend to be costly and thus difficult to implement in the LMIC setting. MATERIAL AND METHODS: A review of the current body of literature on CNS in Africa was conducted using multiple scientific online data bases. Search terms included “CNS tumor registry,” “developing countries,” “low and middle income,” and other related terms as starting point for future initiatives. RESULTS: It was found that more than 1.3 billion people residing in Africa lack access to a continental CTR. There is no well established standards for reporting CNS. Most CNS are still underreported in many countries of Africa. The exact burden of tCNS in Africa is unknown. Although many successful, long-term, initiatives for international neurological and neurosurgical collaborations are published, any CTR of Africa similarly to the Central Brain Tumor Registry of the United States (CBTRUS) is lacking. CONCLUSION: Disparities in access to care for patients suffering from CNS have been well published but well established solutions are still under investigations. Partnerships between centers in LMIC and HIC are making progress to better understand the burden of disease in LMIC and to create context-specific solutions for practice in the LMIC setting. Collaboration between the World Health Organisation, national centers for disease control in Africa, departments of neuroscience in LMIC and well established registries like the CBTRUS as well as other interested groups could be meaningful, because the steps to be taken are the same for the establishment of CTR in Africa. A CTR for Africa could lead to better comprehension of CNS in Africa, thus facilitate prevention, diagnostic, treatment and research.

P13.02.B. FULLY AUTOMATED SEGMENTATION AND VOLUMETRIC MEASUREMENT OF INTRACRANIAL MENINGIOMA USING DEEP LEARNING

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BACKGROUND: Most intracranial meningiomas are small, asymptomatic, and incidentally found tumors. Since the growth of meningioma...
is the principal indication of treatment, accurate and rapid measurement of the volume of intracranial meningiomas is essential in clinical practice to determine the growth rate of the tumor. It could be useful for the management of meningiomas given the increasing incidence of the wait-and-see policy currently in use for asymptomatic meningiomas. The aim of this study was to develop and validate a computational model for fully automated meningioma segmentation and volume measurement based on enhanced MRI scans using deep learning approaches.

**MATERIAL AND METHODS:** The retrospectively collected axial contrast-enhanced T1-weighted section images from patients diagnosed with meningioma were manually segmented and used to construct automatic segmentation models with six U-Net- and nnU-Net-based architectures. The performance of each model was evaluated with the Sørensen-Dice similarity coefficient (DSC) with internal (IVS) and external validation sets (EVS), each consisting of 100 independent MRI examinations. RESULTS: A total of 12,909 section images from 435 patients were applied for the training process. In images with a median age of 58 [52-66] [IQR]: 385 women [83.9%]). The median tumor volume of the training set was 2.36 cm³. A 2D nnU-Net showed the highest median DSCs of 0.922 and 0.893 for the IVS and EVS, respectively. The nnU-Nets achieved superior performance in meningioma segmentation than the U-Nets. The DSCs of the 2D nnU-Net for small meningiomas less than 1 cm³ were 0.769 and 0.780 with the IVS and EVS, respectively. CONCLUSION: We successfully developed a fully automated and accurate volumetric measurement tool for meningiomas using enhanced MRI volumes for small meningiomas, which was significantly better than that achieved in previous studies. The results of this study are clinically applicable and are expected to be of great use in the management of monitored meningioma patients.

**P13.03.A. RADIOMICS FOR THE NON-INVASIVE ASSESSMENT OF THE PDL-1 EXPRESSION IN PATIENTS WITH NON-SMALL-CELL LUNG CANCER WITH BRAIN METASTASES**

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BACKGROUND: Brain metastases were manually segmented on preoperative T1-weighted section images from patients diagnosed with meningioma. A total of 435 patients were included in the study. CONCLUSION: We successfully developed a fully automated and accurate volumetric measurement tool for meningiomas using enhanced MRI volumes for small meningiomas, which was significantly better than that achieved in previous studies. The results of this study are clinically applicable and are expected to be of great use in the management of monitored meningioma patients.

**P13.03.B. DISSECTING HIGH-GRADE GLIOMA IMMUNE INFILTRATION IN SAMPLES FROM FLUORESCENCE-GUIDED SURGERY: DIGITAL PATHOLOGY WITH AUTOMATED IMAGE ANALYSIS**


BACKGROUND: Fluorescence-guided surgery with 5-aminolevulinic acid (5-ALA) is a widely used technique to conduct maximum safe resection of high-grade gliomas (HGG). 5-ALA accumulates in malignant tumor tissue where it is metabolized to Protoporphyrin IX (PpIX), an agent with fluorescence properties. It helps neurosurgeons to distinguish between tumor-infiltrated tissue and healthy brain parenchyma. Even though fluorescence-guided surgery is clinically well established, the biological properties of different fluorescence levels are still not completely understood. A relevant current gap in that respect is the pattern of immune cell infiltration in fluorescent versus non-fluorescent tissue samples. In light of future research, reliable, standardized histopathology methods that allow high-throughput analysis are desirable and digital pathology with automated image analysis is an attractive option to explore. MATERIAL AND METHODS: 128 formalin-fixed paraffin-embedded (FFPE) tissue blocks of 39 patients who underwent fluorescence-guided surgery of a HGG were included on the basis of representative samples for small meningiomas, which were annotated by 4 medical data annotators (T4). A total of 512 stained slides were then available for assessment. In addition to a classical, semi-quantitative analysis by two independent human reviewers, the immune infiltration intensity was measured via automated image analysis with the AGL imaging framework. The comparison of the two analytical methods was the same. Quantitative automated digital pathology correlated well with the classical human histopathological analysis for the majority of markers evaluated. CONCLUSION: We successfully explored and established novel digital pathology technologies for the study of immune cell infiltration patterns in neurooncology, specifically in the context of fluorescence-guided resection. Leveraging this platform could allow parallelized and high-throughput analysis of immune cell infiltration also in other contexts.

**P13.04.A. IMAGE ANNOTATION GUIDELINE FOR INVIVO CONFOCAL LASER ENDOMICROSCOPY, INTERRATER RELIABILITY AND HOW TO LEARN FROM MEDICAL CONSENSUS FOR MACHINE LEARNING ALGORITHM DEVELOPMENT**

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BACKGROUND: Intraoperative confocal laser endomicroscopy (CLE) is an in vivo imaging technique increasingly studied in neurosurgery and neuropathology. It can be affected by artifacts introduced by the CLE device or related to the intraoperative setting. We developed and evaluated an image annotation guideline (AGL) to detect and eliminate images bearing no valuable information as a result of such artifacts. Images were classified into good and bad quality, based on defined technical criteria, which are also considered relevant by clinical experts. MATERIAL AND METHODS: Datasets were created from intraoperative CLE in vivo specimens of patients resected for brain tumors. The process from data collection to development of the ML algorithm followed 7 steps: data quality specification, image and metadata collection, ML development, annotation, data allocation for clinical validation, clinical validation, and, optionally, algorithm development. Final diagnoses were obtained by pathological analysis. Artifacts were grouped into three categories: diminished signal-to-noise-ratio (dSNR), optical distortions, and tissue artifacts. Images were annotated by 4 medical data annotators (T4). For clinical validation, 500 images were excluded from the training data and additionally annotated by 3 board certified neuropathologists (NPs 1-3) with experience in CLE imaging, to determine the medical consensus on good and bad images. Artifacts (NPs) were compared against T4, T4 was