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also compared against the medical consensus. Cohen's Kappa and overall percentage agreement (OPA) were used to evaluate inter-rater reliability. Positive percent agreement (PPA) and negative percent agreement (NPA) were also used to evaluate agreement between medical consensus and T4. RESULTS: 21,616 CLE images and corresponding clinical metadata were collected from 94 patients and annotated. For each case between 27 and 815 CLE images were acquired over the course of the surgery (mean=175 images). T4 had 74% (N=170) of images flagged as dsNR and distortion, and 34% as class contrast. 42% of the images represented the good quality images. Inter-rater agreement between the 3 NPs ranged between 0.30 and 0.39. Agreement between T4 and the medical consensus was substantial (Cohen's Kappa =0.61), OPA between T4 and the medical consensus was 80.60%, PPA 72.34% and NPA 87.92%. CONCLUSION: Annotations according to a well-structured and expertly curated AGL show higher values for Cohen's Kappa and Overall Percent Agreement (OPA) with the medical consensus, than that of individual experts among the others. Such an AGL can be considered appropriate and produces on par results with annotations by a group of experts in the field and can be further employed for training machine learning (ML) algorithms.

P14 LIQUID BIOMARKERS

P14.01.B. ISOLATION AND CHARACTERIZATION OF CIRCULATING TUMOR CELLS IN A Glioblastoma CASE WITH RECURRENCE AT DISTANCE AND CORRELATION WITH TUMOR MUTATIONAL STATUS

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BACKGROUND: Circulating Tumor Cells (CTCs) are considered to be one of the important causes of tumor recurrence and distant metastasis. For many years, glioblastoma (GB) was thought to be restricted to the brain. Nevertheless, a growing body of evidence indicates that, like many other cancers, hematogenic dissemination is a reality. The absence of a procedural uniformity in literature prompted us to develop an innovative and sensitive method to obtain CTCs in GB. Our aim is to define the genetic background of single CTCs compared with the primary GB tumor and its recurrence to assess whether or not their presence in the peripheral circulation correlates with GB migration and dissemination. MATERIAL AND METHODS: CTCs were enriched from whole blood of one patient with recurrent GB with Parsortix Cell Separation System and analysed on DEPArray system. After that, CTCs Copy Number Alterations (CNAs) and sequencing analysis was performed to compare CTCs genetic background with the same patient’s primary and recurrence tissues, analysed by NextSeq 500 (whole exome sequencing). RESULTS: We obtained 211 mutations in common between primary and recurrence tumor. Among these, three somatic mutations (c.430 G > A in PRKCB gene, c.815 C > T in TBX1 gene and c.1554 T > G in COGS gene) were selected to investigate their presence in recurrence and the CTCs of the whole exome sequencing of CTCs (9/13) had at least one of the mutations tested. CONCLUSION: In confirmation of the hypothesis, the CTCs detected in the patient’s blood were actually cancer cells deriving from GB tumor.

P14.02.A. SERUM NEUROFILAMENTS CORRELATE WITH AXONAL LOSS IN PACLITAXEL-INDUCED PERIPHERAL NEUROTOXICITY

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BACKGROUND: Paclitaxel-induced peripheral neuropathy (PPIN) is a dose-limiting axonal polyneuropathy. Nerve conduction studies (NCS) are unique for the assessment of axonal impairment in PPIN. Neurofilaments (NFs), constituent of the neuronal cytoskeleton, are emerging as axonal damage biomarkers. In the present study, the association between changes in NF-L quantification across paclitaxel treatment in breast cancer patients has not been specifically addressed. MATERIAL AND METHODS: Prospective longitudinal measurement of sNfL levels and NCS in 27 chemotherapy-naïve breast cancer patients before (V0) and after completion (V1) of 12 cycles of weekly paclitaxel-based therapy. RESULTS: Serum NfL levels (in pg/ml) increased over the course of treatment in the whole of patients (T0: 18.5±10.5 vs T1: 12.86±7.65 vs T1.5: 19.14±15.52, p<0.017). A significant increase in sNfL levels at T1 showed a significant moderate correlation with the percentage decrease in the amplitude of the sural (r =0.6205 (p=0.0035), radial (r=0.6134, p=0.0035)), ulnar (r =0.6298 (p=0.0035)) and peroneal (r =0.6825 (p<0.001)) nerves in NCS across treatment. CONCLUSION: sNfL levels increase proportionally to the degree of paclitaxel-induced neuroaxonal damage, revealing a promising biomarker able to detect the severity of large nerve fiber degeneration and, therefore, improving the diagnostic accuracy in PPIN.

P14.03.B. GLUTAMATE EXCITOTOXICITY IN BRAIN METASTASES FROM LUNG, BREAST, AND MELANOMA TREATED WITH STEREOTACTIC RADIOSURGERY

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BACKGROUND: Brain metastases (BM) are the most frequent neoplasm in the central nervous system (CNS) and primary tumors frequently involved are melanoma, lung cancer and breast cancer. CNS localisation is associated with worse prognosis, and stereotactic radiosurgery (SRS) represents a treatment option for patients with a good performance status. Glutamate (Glu) is a neurotransmitter which plays a facilitating role in carcinogenesis and progression of malignant tumors, as well as in excitotoxicity. Glu efflux from the brain is regulated by the brain glutamate carrier (GLT-1). Glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH), with aspartate and lactate as catalytically. Glu efflux from the brain seems to be impaired in advanced-stage cancers, resulting in increased blood Glu levels where scavengers exert a protective role. Our hypothesis is that serum Glu and scavengers’ levels are related to neuroinvasion and treatment response in patients with BM and may represent potential biomarkers for BM course and prognosis. MATERIAL AND METHODS: Serum Glu scavengers (GOT1, GPT and LDH), serum Glu, aspartate and lactate levels are collected in included patients treated and grouped in A) BM group of patients affected by BM from lung or breast cancer or melanoma, treated with SRS; B) Control-1 group of patients affected by lung cancer, breast cancer or melanoma but without BM and C) Control-2 group of patients with benign intracranial lesions (meningiomas, acoustic schwannomas) treated with SRS. In A) and C) serum metabolites and scavengers will be analyzed before and after SRS treatment (at 3, 6, 9 months) while in B) analyzed once. Blood levels in A) and C) help in identifying differences related to malignancy, role of SRS and the association with disease control, while blood levels in A) and B) help in detecting differences related to BMs. Exclusion criteria are surgical or previous radiosurgical treatment for BM. This study has received Institutional Ethical Committee approval on 3rd August 2020 (Project NCH04-2020, Clinicaltrials.gov identifier: NCT04785521). PRELIMINARY RESULTS: Comparison between BM group (n = 32) and Control-1 (n =18) revealed a significant difference in LDH (271.93 vs 217.56 U/L; p = 0.006) and glutamate levels (1.86 vs 1.34 mM/L; p = 0.023) and a trend towards significance in glutamine (103.45 vs 73.74 μM/L, p = 0.07). Comparison between BM group (n=32) and Control-2 (n = 37) revealed a difference in LDH (271.93 vs 210.89 U/L; p < 0.001), lactate (1.86 vs 1.24 mM/L; p < 0.001), aspartate (16.36 vs 10.22 μM/L, p = 0.006) and glutamate levels (123 vs 103 μM/L, p = 0.004). CONCLUSION: The present study is the first one addressing serum glutamate and scavenger levels in patients with BM. If the hypothesis will be confirmed, new targets in glutamate signalling pathway could be identified to define new therapeutic strategies in this challenging disease.

P14.04.A. TRACKING TERT PROMOTER AND IDH1 MUTATIONS IN LIQUID BIOPSY - SUITABLE BIOMARKERS FOR DISEASE MONITORING IN GLIOMA PATIENTS

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BACKGROUND: Mutations within the telomerase reverse transcriptase promoter (TERTprom) and its cognate dehydrogenase (IDH) account for the most common genetic alterations in gliomas. Each of these mutations impacts clinicopathologic diagnosis and course of diseases. While TERTprom mutations are frequently detected in glioblastoma, IDH mutations are assigned to astrocytoma of grade 2-4, thus mostly associated with better prognosis. In the era of precision oncology, molecular profiling and continuous monitoring of treatment response or relapse are of increasing importance. Accordingly, this study aims to detect TERTprom and IDH mutations in plasma-derived cell-free (cf)DNA of gliomas. The mutant allele frequencies (MAF) will be compared retrospectively to clinico-pathological parameters including extent of resection and tumor progression. MATERIAL AND METHODS: Digital droplet PCR (ddPCR) analyses were performed using the QX200TM Digital Droplet System from BioRad. First, to evaluate probes for ddPCR, genomic DNA of several brain tumor cell models (n=6) and tumor tissue (n=1), as well as cfDNA of plasma (n=3) from samples with known TERTprom and IDH mutation status was investigated. For detection of IDH mutations, the unique assay ID Hs01000000922 (IDH1-R132H) and for TERTprom mutations the TaqMan ddPCR Liquid Biopsy Assay for C228T and C250T (Hs000000922) were used. The results of ddPCR were analyzed with QuantaSoftTM software and the MAF was calculated RESULTS: To validate the detection method for IDH1R132H, we analyzed the MAF in one tissue and corresponding plasma samples. In a confirmed astrocytoma of grade 2-3 tumor, one IDH1-mutated astrocytoma was tested. Interestingly, both astrocytoma cases exhibited undetectable or very low MAF ranging from 0.1 to 1% as well as in plasma samples, when in all cases, the high grade glioblastoma case from GBM was detected with a frequency of 1.9%. Due to the high GC content of the TERT promoter region, amplification steps are challenging. Accordingly, we first optimized ddPCR conditions for C228T and C250T by adding 7-deaza-2-deoxyguanosine-5-triphosphate (7-ddGTP) in varying concentrations to each ddPCR reaction. When using 4µM of 7-ddGTP per sample, a clear separation between mutant and wild-type droplets was reached, detecting MAF between 36-63% in DNA from cell culture models. CONCLUSION: Within this pilot study we optimized the ddPCR method for the detection of IDH1R132H and TERTprom mutations in plasma and tissue samples. Subsequently, we hypothesize that these mutations are suitable liquid biomarkers correlating with extent of resection and tumor progression in gliomas.

P14.05.B. PLASMA-EV BASED LIQUID BLOOD DISEASE MEDICINE IN THE TREATMENT OF GIBLASTOMA
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BACKGROUND: Glioblastoma (GBM) is a devastating and protein brain tumor. Diagnosis and molecular characterization rely on magnetic resonance imaging and brain biopsy which are challenged by low sensitivity/sensitivity and surgical risks. This hampers the longitudinal monitoring of patients with GBM, whose molecular dynamics ideally requires to refine the understanding of different diseases (EDs) shed by GBM act as a reservoir of circulating biomarkers and can be easily collected through a blood sample, placing the rational to exploit them as a platform for liquid biopsy in GBM. MATERIAL AND METHODS: We isolated EVs from the high-grade glioblastoma case and five GBM patients. EV-RNA length (≤ 200nt) and total yield (1-10ng among different samples) allowed a successful RNAseq library preparation for downstream identification of splicing isoforms, translocations, fused mRNAs, and expression level of GBM-specific transcripts. The ddPCR-based analysis of mutations and copy number alterations proved the ability of cell-derived EV-DNA to recapitulate the alterations of the parental cells. Although the yield of plasma EV-DNA is low (around 1ng), this pathway can pave the way to optimize the isolation/analysis of plasma EV-DNA to permit tumor profiling. CONCLUSION: Plasma EV-based liquid biopsy could implement the personalisation of GBM care for every timepoint of the disease course. Increased plasma EV-concentration is an effective biomarker for GBM presence and its monitoring could complement routine clinical procedures. EV-DNA allows an early genomic profile of parental tumor and their analysis could allow the identification of actionable molecular alterations and the monitoring of GBM mutability during follow-up. Thus, the multi-layered analysis of plasma EV-concentration and cargo could enable GBM early diagnosis and to monitor therapy response and the whole tumor evolution.

P15 DEVELOPMENT AND VALIDATION OF INNOVATIVE IMAGING STRATEGIES (PET, METABOLIC MRI)

P15.01.A. METABOLIC IMAGING OF HUMAN GIBLASTOMA EXPANTS: A NEW PRECISION-MEDICINE MODEL TO PREDICT TREATMENT RESPONSE EARLY
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BACKGROUND: Glioblastoma (GB) is the most severe form of brain cancer, with a 12-15 month median survival. Although cell therapies for GB are on the near horizon, surgical resection, temozolomide (TMZ) and radiotherapy (RT) remain the primary therapeutic options for GB, and no new small molecule therapies have been introduced in recent years. This therapeutic standstill is partially because preclinical models of GB do not reflect the complexities of GB cell biology. Furthermore, the aggressive progression of GB makes it critical to identify patient-tailored therapeutic strategies early. MATERIAL AND METHODS: We developed a novel in-vitro 3D glioblastoma explants (GB-EXPs) model derived from patients’ resected tumors maintaining cytoarchitecture seen in the tumors. We then performed metabolic-imaging by fluorescence lifetime imaging microscopy (FLIM) on live GB-EXPs to predict drug response, using TMZ as test drug. RESULTS: The entire process was successfully completed within 1 week since surgery. A unique drug response sample stratification emerged that was well reflected at the molecular level, highlighting new targets associated with TMZ treatment and identifying a molecular signature associated with survival. CONCLUSION: To test anti-neoplastic drugs, FLIM-based readouts of drug response in GB explants could accelerate precision treatment of patients with GB and the identification of next anti-GD drugs.

P15.02.B. SPATIAL HABITAT ANALYSIS IN HIGH-GRADE GLIOMAS: COMBINING PERSEUS, DIFFUSION AND HYPOXIA FEATURES DERIVED FROM MULTIPARAMETRIC MRI AND 18F-FAZA PET
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BACKGROUND: Heterogeneity represents one of the main issues in high-grade gliomas (HGGs) management and presumably is the key to understanding treatment failure. Spatial habitat imaging embodies a novel, non-invasive diagnostic method to characterize tumoral and microenvironmental heterogeneity and characterize intratumoral heterogeneity through a quantitative radiomic approach. So far, habitat imaging has been chiefly explored on morphological magnetic resonance imaging (MRI): the aim of our study was to evaluate this technique by using multi-parametric imaging (MRI and PET). MATERIAL AND METHODS: A preoperative PWI, dMRI, and 18F-FAZA PET acquisition was obtained in 17 HGG patients (GB-EXPs) model derived from patients’ resected GBM. We analyzed FLIM-based metabolic imaging on live glioblastoma explants to predict drug response, using TMZ as test drug. RESULTS: The entire process was successfully completed within 1 week since surgery. A unique drug response sample stratification emerged that was well reflected at the molecular level, highlighting new targets associated with TMZ treatment and identifying a molecular signature associated with survival. CONCLUSION: To test anti-neoplastic drugs, FLIM-based readouts of drug response in GB explants could accelerate precision treatment of patients with GB and the identification of next anti-GD drugs.