to the WHO classification. The diagnosis is based on cranial MRI, in combination with a brain biopsy. In case of classical MRI findings, the identification of lymphoma cells in the cerebrospinal (CSF) or vitreous fluid by cytology and flow cytometry might obviate brain biopsy. The presence of the somatic mutation p.Leu265Pro (L265P) in MYD88 is detectable in 50 to 80% of PCNSL, and might also be helpful to confirm the diagnosis. The aim of our study was to evaluate the contribution of a highly sensitive digital droplet PCR, targeting the mutation L265P MYD88, for the detection of tumoral circulating DNA from CSF supernatant. **MATERIAL AND METHODS:** We identified 9 PCNSL expressing the L265P mutation at diagnosis or relapse. The mutation was found by an allele specific PCR technique either on biopsy or in CSF cells. Circulating DNA was isolated from CSF supernatant with the « QiaAmp® circulating nucleic acid» kit. The quantity of DNA collected was estimated by quantitative PCR for a reference gene (albumine) with 7900HT (Life technologies™) device. Subsequently, the L265P MYD88 mutation was quantified by digital droplet PCR Biorad™: The droplets generated were amplified by PCR, detected with the QX200 Reader, and analyzed with the QuantaSoft™ software. **RESULTS:** The circulating DNA concentration was low, varying between 0 and 2.2 ng/mL of CSF. However, the mutation was detected in the circulating DNA from CSF supernatant in 6 out of 9 cases (66%). The fractional abundance varied from 2.6 to 85%. In 3 cases, the mutation was detected even though cytology and flow cytometry did not reveal leptomeningeal disease. For 3 other cases, the mutation was not detected: The genome copy number was below 1 copy/µL, indicating a low analytical sensitivity for theses samples. **CONCLUSION:** This study shows that circulating DNA is present in low concentration in CSF and can be amplified by a sensitive digital PCR for the L265P MYD88 mutation. The detection of circulating PCNSL DNA in CSF is possible and might be used to improve the non-invasive diagnosis of PCNSL. It might also help to select patients for targeted therapies.

OS1.4 LIQUID BIOPSY OF THE CSF IN A SERIES OF GBM PATIENTS: PRELIMINARY RESULTS

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BACKGROUND: Liquid biopsy (LB) by cerebrospinal fluid (CSF) can be useful to identify circulating tumour DNA (ctDNA), thus offering information about the heterogeneity of the neoplastic genome. The aim of our study is to assess the effectiveness of LB of the CSF in detecting ctDNA which mirrors the genetic profile of the tumoural tissue, and to investigate the clinical and radiological aspects influencing the availability of ctDNA. **MATERIAL AND METHODS:** Tumoral tissue and CSF samples of 13 GBM patients undergoing surgery was collected. CSF was withdrawn from the very proximity of the tumoural surface before the excision. DNA extracted from tissue samples was analysed by qPCR to identify typical genetic alterations such as copy number variations (EGFR, PDGFRA, CDK4, MDM2, CDKN2A), and point mutations (TP53, PTEN, IDH, NRAS, PI3K1, pTERT). CtDNA extracted from CSF was analysed by droplet digital PCR to assess the presence of the alterations found in the matching tissue. Both contrast-enhanced (CE) and FLAIR volumes of the lesions were measured in the pre-surgical MRI. Linear and logarithmic regressions were employed for the statistical analysis. **RESULTS:** From June 2016 to February 2017 we prospectively collected 13 GBM patients. Median age was 73 years. All lesions showed CE at the MRI; other radiological findings included necrosis (84.6%), oedema (76.9%), cortical, ventricular or meningeal involvement (76.9%, 30.8%, and 15.4%). Median volumes of CE and FLAIR lesions were 28.6 and 25.5 cm3, with a median FLAIR/CE ratio of 72.9. Surgery was subtotal (<95%) in all patients. All GBM tissues were tested for the following alterations: EGFR, PDGFRA, CDK4, MDM2, CDKN2A; 76.9% were tested for TP53, PTEN, and IDH mutations; 38.5% for NRAS and pTERT mutations; 30.8% for PI3KR1 mutation. MGMT methylation was assessed in 12 cases (92.3%) and found in 7 (58.3%). Median CSF volume, ctDNA quantity and concentration were 0.45 mL, 59.64 ng, and 0.42 ng/µL. Processable DNA was found in 11 CSF specimens (84.6%), in 8 of which (61.5%) it carried the same alteration expressed by the tumoural cells of the matched tissue, while in 3 cases (23.1%) it seemed to have a different genetic profile; finally, in 2 cases it was not possible to detect any circulating DNA in the CSF. Preliminary data on 13 patients suggest that the ctDNA concentration in the CSF could be related to the FLAIR/CE ratio as measured in the MRI before surgery ($p = 0.02$). Other correlations between the molecular and the radiological features are still being exploring. **CONCLUSION:** Our study confirms that LB of CSF can detect ctDNA carrying the same molecular profile harboured in the tumour. Therefore, it seems to be an accurate method to identify markers useful for the diagnosis and the monitoring of the disease. Additionally, our ongoing study is trying to demonstrate a potential correlation between radiological features of the tumour and availability of ctDNA in CSF.

OS1.5 DETECTION OF GLIOMA AND PROGNOSTIC SUBTYPES BY NON-INVASIVE CIRCULATING CELL-FREE DNA METHYLATION MARKERS

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BACKGROUND: Genome-wide DNA methylation profiling has shown that epigenetic abnormalities are biologically important in glioma and can be used to classify these tumors into distinct prognostic groups. Thus far, DNA profiling has required surgically resected glioma tissue; however, gliomas release tumoral material into biofluids providing an opportunity for a minimally invasive testing. While prior studies have shown that molecular markers can be detected in liquid biopsy (LB), there has been low sensitivity for tumor-specific markers. We hypothesize that the low sensitivity is due to the targeted assay methods. METHODS: Genome-wide CpG methylation levels in DNA of tumor tissue and cell-free DNA serum of glioma patients. RESULTS: We defined glioma-specific and IDH-specific epigenetic LB (eLB) signatures (Glioma-eLB and IDH-eLB, respectively) from serum cell-free DNA from patients diagnosed with glioma (N=15 IDH mutant and N=7 IDH wildtype) and with epilepsy (N=3). The epigenetic profiles of the matched tissue demonstrate that these eLB signatures reflected the signature of the tumor. Through cross-validation we show that Glioma-eLB can accurately predict a patient's glioma from those with other neoplasias (N=6 Colon; N=14 Pituitary; N=3 Breast; N=4 Lung), non-neoplastic immunological conditions (N=22 sepsis; N=9 pancreatic islet transplantation), and from healthy individuals (sensitivity: 98%; specificity: 99%). Finally, IDH-eLB includes promoter methylated markers associated with genes known to be involved in glioma tumorigenesis (PVT1 and CXCR6). CONCLUSIONS: The application of the non-invasive eLB signature discovered in this study has the potential to complement the standard of care for patients harboring glioma.

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OS1.6 PERIPHERAL BLOOD IMMUNE PROFILES AT FIRST RECURRENCE OF IDH WILD TYPE GLIOBLASTOMA AFTER STANDARD CHEMORADIOTHERAPY PREDICT OVERALL SURVIVAL: SECONDARY ANALYSES OF THE PHASE II DIRECTOR TRIAL

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BACKGROUND: Isocitrate dehydrogenase (IDH) wildtype glioblastoma is associated with distinctive peripheral blood immune cell profiles that evolve under first line chemoirradiation with temozolomide. Whether peripheral blood immune cell profiles at recurrence are associated with survival of IDH wildtype glioblastoma has not been studied in detail. **PATIENTS AND METHODS:** Peripheral blood mononuclear cells (PBMC) of 21 healthy donors and of 52 clinically well-annotated patients with IDH wildtype glioblastoma were analyzed by 11-color flow cytometry at 1st recurrence after standard chemoirradiation with temozolomide and at 2nd recurrence after dose-intensified temozolomide re-challenge. Patients were treated within the randomized phase II trial DIRECTOR, which explored the efficacy of dose-intensified temozolomide at first recurrence of glioblastoma. Patients were classified based on unsupervised analyses of PBMC profiles at 1stand 2ndrecurrence. Associations with survival were explored in multivariate Cox models controlling for established prognostic and predictive factors. **RESULTS:** At 1strecurrence, two patient clusters were identified which differed in CD4+ T-cell fractions, but not with respect to CD8+ T-cells, CD4+;CD25+;FoxP3+ regulatory T-cells, B-cells or monocytes. The composition of CD4+, CD8+ or regulatory T-cell fractions was similar in both clusters. All control samples clustered with the CD4high cluster. Patients in both clusters did not differ by established prognostic factors, including age, O6-methylguanine-DNA-methyl-transferase (MGMT) gene promoter methylation, tumor volume, Karfnosky performance score or steroid use. Progression-free survival was similar ($CD4^{high}$ vs$CD4^{low}$ 2.1