M. Alonso[1], J. Gállego Pérez-Larraya[1]; [1]Clinica Universidad de Navarra. CIMA, Pamplona, Spain, [2]Complejo Hospitalario de Navarra, Pamplona, Spain, [3]Hospital de Bellvitge, Barcelona, Spain, [4]Hôpital de la Timone, Marseille, Spain, [5]Clinica Universidad de Navarra., Pamplona, Spain, [6]Research support service. Clínica Universidad de Navarra., Pamplona, Spain.

BACKGROUND: Glioblastoma (GBM) is the most common malignant primary brain tumour in adults, and is still associated with a dismal prognosis despite intensive research and therapeutic efforts. The identification of circulating biomarkers for tumor detection and response assessment would be of great clinical help. Recent studies have shown that GBM cells release microvesicles containing a select subset of cellular proteins and RNA. Previously, we demonstrated that exosomes isolated from the serum of GBM patients had an increased expression of the small non-coding RNA RNU6-1 compared to control samples, and hence could serve as a non-invasive diagnostic biomarker for GBM.In this study, we set to investigate the role of RNU6-1 as a differential biomarker of GBM versus other brain diseases with similar radiological features. MATERIAL AND METHODS: We analyzed the expression of RNU6-1 in circulating exosomes of GBM patients (n=18), healthy controls (n=28), and patients with different brain lesions that can mimic GBM on Magnetic Resonance Imaging: subacute stroke (n=30), acute-subacute haemorrhage (n=29), acute demyelinating lesions (n=19), brain metastases (n=21) and Primary CNS Lymphomas (PCNSL) (n=12). First, we isolated the exosomes from the serum of healthy subjects and patients with these pathological conditions using an adsorption method (Exoquick). Then, RNU6-1 levels were analyzed by digital droplet PCR (ddPCR). RESULTS: Corroborating our previous results, we found that the expression of RNU6-1 was significantly higher in GBM patients (412 ± 550.48 copies/20µL) than in healthy controls (150 ± 224.35 copies/20µL; p=0.039). In addition, RNU6-1 levels were higher in exosomes from GBM patients than in exosomes from patients with non-neoplasic lesions (stroke [223 ± 709.8 copies/20µL; p=0,067], haemorrhage [127 ± 198.7 copies/20µL; p=0.010], demyelinating lesions [111.5 ± 250.35 copies/20µL; p=0.019]) and PCNSL [18.15 ± 245.7 copies/20µL; p=0.004]). In contrast, RNU6-1 levels were similar between brain metastases and GBM patients [325 ± 632 copies/20µL; p=0.573]. In addition, analyzing whether this small non-coding RNA could be a predictive marker of GBM by ROC curves analysis, we demonstrated that RNU6-1 was a robust diagnostic biomarker of GBM compared to subacute stroke [AUC=0.659; p=0.004], acute/subacute haemorrhage [AUC=0.724; p=0.006], acute demyelinating lesions [AUC=0.728; p=0.011] and PCNSL [AUC=0.814; p<0.001]; on the contrary, it did not allow differentiating GBM from brain metastases [AUC=0.552; p=0.575]. CONCLUSION: Our data suggest that RNU6-1 isolated in circulating exosomes could serve as a differential biomarker for GBM versus non-neoplastic brain lesions and PCNSL.(Grant: 42/2015 Dpto. de Salud Gobierno de Navarra)

OS1.2 STABILITY OF *EGFR* AMPLIFICATION IN GLIOBLASTOMA IS DIFFERENTIALLY IMPACTED BASED ON THERAPEUTIC PRESSURE

M. Ahluwalia[1], Y. Narita[2], Y. Muragaki[3], H. Gan[4], R. Merrell[5], M. van den Bent[6], L. Roberts-Rapp[7], M. Guseva[7], P. Ansell[7], A. Lassman[8]; [1]Burkhardt Brain Tumor and Neuro-Oncology Center, Cleveland Clinic, Cleveland, OH, United States, [2]National Cancer Center Hospital, Tokyo, Japan, [3]Tokyo Women's Medical University Hospital, Tokyo, Japan, [4]Austin Health and Olivia Newton-John Cancer Research Institute, ClevelandMelbourne, Australia, [5]NorthShore University HealthSystem, Evanston, IL, United States, [6]Erasmus MC Cancer Centre, Rotterdam, Netherlands, [7]AbbVie Inc., North Chicago, IL, United States, [8]Columbia University Medical Center, New York, NY, United States.

BACKGROUND: Depatuxizumab mafodotin (depatux-m, formerly ABT-414) is an EGFR-directed antibody-drug conjugate being developed for treatment of *EGFR*-amplified glioblastoma (GBM). As therapeutic pressure engenders tumor adaptations, it is important to understand the stability of biomarkers targeted by precision medicine approaches such as depatux-m. Therefore, we assessed *EGFR* amplification (amp) and expression in longitudinally-sampled GBMs from patients (pts) treated +/- depatux-m to explore biomarker stability. MATERIAL AND METHODS: Formalin-fixed, paraffin embedded GBM tumor tissue was analyzed from 68 patients who underwent at least 2 surgeries; *EGFR* amp was detected by *in situ* hybridization (*e.g.*, FISH) or next-generation sequencing in all samples. Fifty-six pts did not receive depatux-m; among 12 pts who did, *EGFR* expression was also evaluated by RNA sequencing. RESULTS: Of 56 pts who did not receive depatux-m, 31 (55%) had tumors harboring EGFR amp at 1st surgery (initial diagnosis); among those, EGFR amp was maintained at re-operation in 27 (87%), and not maintained in 4 (13%). None of the 25 cases without baseline EGFR amp acquired it at the 2nd surgery. Of 12 pts treated with depatux-m between surgeries, 9 cases harbored *EGFR* amp at baseline which was maintained in 4 (44%) at the 2nd surgery, all 4 of which had the highest levels of *EGFR* expression at baseline. Of the 3 cases without *EGFR* amp at baseline, none acquired amplification at the 2nd surgery, and all 3 had the lowest levels of *EGFR* expression at study start. CONCLUSION: The presence of *EGFR* amp in GBM tissue at baseline was maintained at 2nd surgery in 87% of pts who received treatment other than depatux-m, and in 44% following depatux-m exposure. Therefore, depatux-m exposure appears to reduce *EGFR* amp maintenance (p=0.0159 by Fisher's exact test); accordingly, the therapeutic approach may influence EGFR status. In no case was *EGFR* amp acquired at recurrence, regardless of depatux-m therapy. Ongoing analyses of additional tumor samples will increase power and further examine concordance among *EGFR* amp assays.

OS1.3 CLINICAL SIGNIFICANCE OF PLASMA EVS IN GLIOBLASTOMA PATIENTS

M. Del Bene[1,2], D. Osti[2], G. Rappa[3], M. Santos[3], V. Matafora[4], C. Richichi[2], S. Faletti[2], G. Beznoussenko[4], A. Mironov[4], A. Bachi[4], L. Fornasari[2], D. Bongetta[5], P. Gaetani[5], F. DiMeco[1], A. Lorico[3], G. Pelicci[2,6]; [1]Istituto Neurologico C. Besta, Milano, Italy, [2]European Institute of Oncology, Milano, Italy, [3]Roseman University of Health Sciences, Las Vegas, NV, United States, [4]The FIRC Institute of Molecular Oncology, Milano, Italy, [5]IRCCS Fondazione Policlinico San Matteo, Pavia, Italy, [6]Piemonte Orientale University 'Amedeo Avogadro', Novara, Italy.

BACKGROUND: Glioblastoma (GBM) is the most common primary brain tumor. Despite aggressive treatment (surgery, chemo- and radiotherapy), the prognosis is dismal (median overall survival: 14m). Actually, early diagnosis and treatment monitoring represent major unmet needs. Extracellular vesicles (EVs) are potentially optimal biomarkers owing to high stability and their presence in blood and cerebrospinal fluid. These features, combined to their genetic and proteomic composition that mirrors the intratumoral environment, highlight EV applicability as blood-based biomarkers for disease diagnosis and therapeutic monitoring. MATERIAL AND METHODS: We collected plasma samples from healthy controls (n=33), GBMs (n=43) and different central nervous system malignancies (n=25). EVs were isolated and subjected to an integrated analysis relying on transmission electron microscopy, Nanoparticle Tracking Analysis, and mass spectrometry in order to assess morphology, size, concentration and protein composition. An orthotopic mouse model of human GBM was employed to confirm human plasma EV quantifications. Possible associations between plasma EV concentration and clinical features were analyzed. All statistical tests were two-sided. RESULTS: We observed a significant EVs enrichment in GBM patients plasma if compared to healthy control and patients harboring other CNS malignancies. GBM surgical removal was accompanied by a relevant drop in the concentration of circulating EVs followed by a re-increase at the relapse; this indicates the tumor mass as the major donor of circulating EVs in GBM patients. This phenomenon was confirmed by mouse model. The analyses of EV protein cargo revealed a specific signature, which included members of the complement/coagulation cascade and regulators of iron metabolism. CONCLUSION: Our work demonstrates the potential applicability of circulating EVs as reliable cancer biomarker for GBM patients. Furthermore we observed that EV concentration and protein cargo might provide information about response to therapies and tumor progression.

OS1.4 INDUCTION OF MITOTIC CELL DEATH: A NOVEL THERAPEUTIC STRATEGY FOR GLIOBLASTOMA

N. Gomez-Roman, A. J. Chalmers; University of Glasgow, Glasgow, United Kingdom.

BACKGROUND: Glioblastoma (GBM) is the most common and lethal adult brain tumour. Tumours typically contain large numbers of binuclear and multinucleated cells, and a subpopulation of relatively quiescent glioma stem-like cells (GSC) that are thought to be responsible for treatment resistance and tumour recurrence. GSC display robust G2/M arrest following ionising radiation (IR) yet are highly prone to aberrant cell division and are sensitive to mitotic spindle checkpoint inhibitors. The aim of this study was to investigate the therapeutic activity of mitotic inducers in preclinical models of GBM. MATERIAL AND METHODS: Bioinformatic analysis of mRNA expression data was used to confirm the relevance of mitotic activity following irradiation of GSC in a 3D patient-derived GBM stem cell model. Immunofluorescence, clonogenic survival and cell viability assays were used to evaluate the therapeutic potential of mitotic inducers (ME-344; Wee1 inhibitor AZ1775) in 2D and 3D U87MGLuc and E2, G1, G7, S2 and R15 patient-derived GSC cell culture models. either alone or in combination with IR. *In vivo* validation was determined in U87-MGLuc orthotopic GBM mouse model. RESULTS: Radiation induced downregulation of mRNA expression of several mitotic genes was observed in G7 and E2 cell lines confirming mitotic relevance. In cell viability and clonogenic survival assays, AZ1775 and ME-344 showed potent cytotoxicity against all GSC cell lines in both 2D and 3D, with EC50 values of 0.2 to 0.4 µM for AZ1775 and 0.003 to 0.02 µM for ME-344. ME-344 and AZ1775 triggered profound morphological and cell cycle effects including mitotic induction and arrest, increased mitotic fraction, reduced nuclear tubulin in mitotic cells

and, induced mitotic catastrophe in the most sensitive cell lines U87MGLuc and E2. These events were apoptosis-independent. Combination with IR increased GSC cell death in the two GSC models tested to date. Accumulation of cells in mitosis following ME-344 treatment was recapitulated in orthotopic GBM xenografts *in vivo*, although few mitotic catastrophe events were observed 24 h after treatment. ME-344 demonstrated therapeutic efficacy as a single agent in U87MGLuc2 orthotopic xenografts by extending mouse survival compared to vehicle (p=0.043). CONCLUSION: Two agents that induce mitosis through different mechanisms have promising single agent activity against all GBM cell lines tested *in vitro* and *in vivo*. Further preclinical evaluation in combination with IR and/or temozolomide is underway. Results indicate that this therapeutic strategy for GBM has clinical potential.

OS1.5 HARNESSING SOLUBLE LRIG1 FOR PAN-RTK TARGETING IN GLIOBLASTOMA

V. Neirinckx[1], A. Schuster[1], A. Chevigné[1], M. H. H. Schmidt[2,3], S. P. Niclou[1,4]; [1]Luxembourg Institute of Health, Luxembourg, Luxembourg, [2]Johannes Gutenberg University, Mainz, Germany, [3]German Cancer Research Center (DKFZ), Heidelberg, Germany, [4]KG Jebsen Brain Tumour Research Center, Bergen, Norway.

INTRODUCTION: The role of receptor tyrosine kinases (RTKs) in glioblastoma is widely acknowledged. However, therapies based on RTK targeting have been continuously unsuccessful in GBM patients, highlighting the complexity of RTK signaling and biology. LRIG1 (Leucine-rich Repeats and ImmunoGlobulin domains protein 1) was identified as an endogenous inhibitor of epidermal growth factor receptor (EGFR) and other RTKs, and was confirmed as a tumor suppressor in various cancer types. We previously identified the soluble form of LRIG1 as a potent inhibitor of GBM growth *in vivo*, irrespective of EGFR status. Here, we aim to shed light on the molecular mechanisms underlying its anti-cancer activity. MATERIAL AND METHODS: We used GBM cells overexpressing EGFRvIII, with or without soluble LRIG1 overexpression. In parallel, we generated a recombinant human soluble LRIG1 protein (rh-sLRIG1) by expressing LRIG1 ectodomain in insect cells via baculovirus infection and subsequent His-tag purification. rh-sLRIG1 was applied in the medium of classical GBM cell lines and patient-derived GBM stem-like cells. Applying a variety of cell-based assays, cell proliferation, migration, cell morphology, as well as protein expression and protein-protein interactions were investigated. RESULTS: We confirmed that sLRIG1 efficiently reduced proliferation and invasion capacities of GBM cells, and modulated cytoskeleton proteins and cell shape. Inhibition of cell proliferation by sLRIG1 was independent of EGFR expression levels in GBM cells and interestingly, rh-sLRIG1 treatment was associated with downregulation of AXL, which constitutes a newly-identified regulatory function of LRIG1. We are currently addressing the impact of the LRIG1-AXL signaling axis on GBM invasion and resistance to EGFR inhibition. CONCLUSION: We identified AXL as a novel LRIG1 target and provide evidence for the potential therapeutic application of recombinant sLRIG1 in the inhibition of growth factor signaling in GBM.

OS1.6 CHARACTERIZING THE OVER-EXPRESSION OF YKI/YAP/TAZ TRANSCRIPTION FACTORS IN GLIOMAGENESIS AND RESULTS OF A PHASE 0 CLINICAL TRIAL FOR A PROPOSED NOVEL TREATMENT OF GLIOBLASTOMAS

K. Vigneswaran, S. Oh, S. Lallani, R. Read, J. Olson; Emory University School of Medicine, Atlanta, GA, United States.

BACKGROUND: Glioblastomas (GBMs) harbor frequent genetic lesions that include amplification, mutation, and/or over expression of receptor tyrosine kinases (RTKs). Using a novel kinome wide RNAi screen we identified the Hippo kinase pathway and it downstream targets, Yki-YAP/TAZ transcription factors, as tumor enhancers in gliomagenesis. YAP/TAZ promote the initiation and progression of other tumor types and several published studies show that pharmacologic inhibition of YAP and TAZ with the drug verteporfin (VP) blocks tumor cell growth. Thus, we hypothesize that, because of RTK mutations, YAP/TAZ become overexpressed and activated to constitutively drive a TEAD-dependent gene expression program that provokes an uncontrolled expansion of RTK-PI3K mutant neural stem/progenitor cells to create malignant glial tumors. MATERIAL AND METHODS: We tested VP in vitro on genotyped neurosphere cultures which were assessed for self-renewal, proliferation, and survival using neurosphere formation and WST-1 assays. To confirm that VP inhibits expression of YAP/TAZ-TEAD transcriptional targets, we performed experiments and harvested RNA for RNAseq, qPCR, and completed western blots and ChIP analysis. In-vivo experiments were carried out in murine xenografts bearing YAP/TAZ-expressing GBM that were used to make organotypic slice cultures which were treated with VP and assayed for tumor growth and cell survival. A Phase 0 clinical trial was designed to determine VP bioavailability. Because VP has virtually the same excitation and emission spectra as protoporphyrin IX, we administered VP to patients prior to surgery and used fluorescence-assisted microscopy to determine if VP is visible in tumors. On encountering tumor intraoperatively, the operative microscopy system was used to illuminate the tumor bed with blue light (400–410 nm), and photographs were taken through the microscope using a camera adapted for imaging in the red (620–700 nm) emissions spectrum. Resected tumor tissue remaining after satisfying clinical goals was sent for ex vivo research analysis. RESULTS: Our data reveal that YAP and TAZ become overexpressed in tumor cells with RTK mutations, and that YAP/TAZ drive brain tumor cell growth and progression by up-regulation of novel RTK genes including EGFR. VP treatment knocks down target gene transcription, protein levels, leads to cell death and halts tumor progression. VP extraction from tumor tissue and fluoroscopic examination show successful drug uptake from all patients in a Phase 0 clinical trial. CONCLUSION: We believe that as a consequence of RTK mutations, YAP/TAZ becomes over-expressed in gliomas and constitutively drive a TEAD-dependent gene expression program that provokes an uncontrolled expansion of RTK -PI3K mutant neural stem/progenitor cells to create malignant glial tumors that can be treated with VP which shows bioavailability in glioblastomas.

OS1.7 GENOMIC ATTRIBUTES OF TUMOR EVOLUTION AND TREATMENT RESPONSE IN DIFFUSE GLIOMA

A. L. Lin, P. Jonsson, S. Ogilvie, S. Chavan, C. Nolan, I. Gavrilovic, T. Kaley, C. Grommes, E. Pentsova, E. Diamond, M. Daras, J. Stone, L. DeAngelis, V. Tabar, C. Brennan, R. J. Young, M. Rosenblum, B. S. Taylor, I. K. Mellinghoff; Memorial Sloan Kettering Cancer Center, New York, NY, United States.

BACKGROUND: Though the genomic landscape of primary gliomas has been well characterized by The Cancer Genome Atlas, the genetic determinants of malignant transformation and response to therapy remains poorly understood. MATERIAL AND METHODS: Prospective clinical sequencing was performed on 1,004 gliomas from 923 patients. This dataset includes primary and recurrent tumors and contains detailed clinical annotation, including review of the patients' imaging. RESULTS: We investigated the germline and somatic attributes of IDH1/2-wildtype and IDH1/2-mutant tumors at the time of diagnosis and recurrence. 13% of patients harbored either a pathogenic or likely pathogenic germline mutation, whereof 29% arose in genes mediating DNA repair. In astrocytomas, agnostic of IDH status, cell cycle alterations were depleted in low-grade tumors. Moreover, mutations in effectors of the cell cycle were associated with the development of enhancing disease in IDH-mutant astrocytomas but not oligodendrogliomas. IDH-mutant astrocytomas with a cell-cycle alteration have a significantly shorter progression-free survival from recurrence compared to tumors without a cell cycle alteration (median 2. 5 vs. 35. 3 months, HR 3. 25, log-rank p-value 0. 00061). Based on our data, hypermutation appears to occur exclusively in the context of pre-existing cell cycle alterations in astrocytic tumors, regardless of IDH status. We next correlated molecular findings with clinical behavior and treatment response and defined subsets of gliomas that are uniquely susceptible to targeted treatment and have a differential prognosis. CONCLUSION: Cell-cycle alterations are lineage-specific alterations associated with aggressive disease in glioma. Targeted genomic sequencing can identify subsets of tumors with a greater sensitivity to treatment and a better prognosis.

OS2 NON-SURGICAL TREATMENT

OS2.1 OBJECTIVE RESPONSES TO CHEMOTHERAPY IN RECURRENT GLIOMA DO NOT PREDICT BETTER SURVIVAL: A PROSPECTIVE ANALYSIS FROM THE GERMAN GLIOMA NETWORK

O. Bähr[1], B. Hentschel[2], E. Hattingen[3], M. Reusche[2], M. Tatagiba[4], J. Tonn[5], O. Schnell[6], G. Schackert[7], M. Westphal[8], U. Herrlinger[3], T. Pietsch[3], G. Reifenberger[9], M. Weller[10], M. Löffler[2], J. P. Steinbach[1]; [1]University Hopsital Frankfurt, Frankfurt, Germany, [2]University Leipzig, Leipzig, Germany, [3]University Hopsital Bonn, Bonn, Germany, [4]University Hopsital Tübingen, Tübingen, Germany, [5]University Hopsital Munich (LMU), Munich, Germany, [6]University Hopsital Freiburg, Freiburg, Germany, [7]University Hopsital Dresden, Dresden, Germany, [8]University Hopsital Hamburg (UKE), Hamburg, Germany, [9]University Hopsital Düsseldorf, Düsseldorf, Germany, [10]University Hopsital Zurich, Zurich, Germany.

BACKGROUND: Outside of clinical trials, the occurrence of objective responses (OR) to chemotherapy in patients with recurrent gliomas is poorly characterized. Further, the predictive value of OR for progression-free survival (PFS) and overall survival (OS) in glioma patients is unclear. MATERIAL AND METHODS: We screened the German Glioma Network Database for patients who had received any chemotherapy for recurrent glioma from 2004–2008. Patients with a prior gross total resection of the recurrent tumor, patients receiving additional radiotherapy for the recurrent