underlying disease. We reported a girl, with a clinical diagnosis of sporadic NF1 during childhood, who presented a glioblastoma at 16 years-old. Faced with the NF1-like phenotype, diagnosis of CMMRD was suspected because tumor was ultra-hypermutated (228.67 mut/Mb), with a loss of PMS2 expression in both tumor and normal cells. Germline analyses identified a compound heterozygous pathogenic variant (PV) in the PMS2 gene, with an abnormal methylation tolerance test, that confirmed CMMRD. Moreover, a NF1 PV (20% and 9% in blood and saliva samples respectively) was identified compatible with a germline mosaicism. Patient's phenotype was atypical for CMMRD, with a voluminous neurofibroma and ephelids rather observed in NF1. CMMRD oncogenesis is not currently understood, in particular involvement of an NF1 PV, which could arise early from the ultra-hypermutated burden and might explain clinical signs, in particular CALMs. CMMRD diagnosis allowed proposing an adapted genetic counseling and surveillance for the patient and her parents according to the published guidelines due to the major impact on the patient's oncological risks and prognosis. The best strategy for surveillance of the neurofibroma is still debated due to uncertainties about its risk of degeneration with a CMMRD underlying disease. This observation raises the question of the frequency of mosaic NF1 germline PV in CMMRD-patients and the time of its postzygotic appearance in the context of a biallelic deficit of one of the MMR genes. Combination of these both CMMRD and NF1 germline PVs would be a strong argument for a combination of MEK-inhibitors with immunotherapy.

HGG-41. GLIOMA ONCOGENESIS IN THE CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY (CMMRD) SYNDROME

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PURPOSE: Constitutional Mismatch Repair Deficiency (CMMRD) is a cancer predisposition due to bi-allelic mutations in one of the four main mismatch repair (MMR) genes (PMS2, MSH2, MSH6 or MLH1) associated with early onset of cancers, especially glioblastomas (GBM). Our aim was to decipher the molecular specificities of gliomas occurring in this context. METHODS: A comprehensive analysis of clinical, histopathological and genomic data (whole exome sequencing) was performed for 12 children with a CMMRD for which we had available frozen brain tumor material (10 GBM and 2 anaplastic astrocytomas). RESULTS: Eight patients harbored an ultra-mutated phenotype with more than 100 somatic non synonymous (NS) SNV/Mb. No correlation was observed between the number of mutation and sex, age, overall survival or mutated MMR gene. POLE and POLD1 exonuclease domain driver somatic mutations were described for eight and one patients respectively. The 4/12 tumors without POLE somatic mutation did not show the classical ultra-hypermutation pattern. All patients with POLE mutation had already more than 20 NS SNV/Mb (median 40, [range 23-114]) suggesting that the hypermutation phenomenon started before the appearance of the somatic POLE mutation. The mutational signatures of the tumors, dominated by the MMR signatures, were not modified after the onset of the POLE mutation when analyzing the different mutation bursts. Specific recurrent somatic mutations were observed in SETD2 (9/12), TP53 (9/12), NF1 (9/12), EPHB2 (8/12), and DICER1 (7/12). Only half of the tumors overexpressed PDL1 by immunohistochemistry and this overexpression was not associated with a higher tumor mutation burden. CONCLUSION: CMMRD-associated gliomas have a specific oncogenesis that does not trigger usual pathways and mutations seen in sporadic pediatric or adult GBM. Frequent alterations in other pathways (e.g. MAPK or DNA-PK pathway) may suggests the use of other targeted therapies aside from PD1 inhibitors.

HGG-42. EVOLUTIONARY SELECTION OF KEY ONCOGENIC ALTERATIONS IN PATIENT-DERIVED MODELS OF PAEDIATRIC DIFFUSE HIGH GRADE GLIOMA (PDHGG) SUBTYPES *IN VITRO* AND *IN VIVO*

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PDHGG are a diverse group of childhood brain tumours comprising multiple subgroups carrying distinct molecular drivers. Patient-derived models accurately recapitulating this underlying biology are critical for mechanistic/preclinical studies aimed at improving patient outcome, however their behaviour over time in the environments in which they are propagated, and how this relates to the human disease, is largely unknown. To explore this, we collected 94 models of PDHGG established as 2D/3D stem cell cultures in vitro, and generated patient-derived xenografts (PDX) in 33/62 specimens implanted orthotopically in vivo. We carried out exome/targeted sequencing, methylation profiling and RNAseq to profile cells through their first 25 passages in culture, and sequential implantation from p0-p2 in mice. In 15/83 cultures, we observed enrichment of gene expression signatures of non-malignant cells over the first 5 passages, with concurrent depletion of somatic mutations/CNAs, excluding them from further study. Validated models retained tumour-matched genotypes, CNAs and driver alterations including H3.3G34R, H3.3/H3.1K27M, BRAF and ACVR1 over time, however subclonal alterations underwent selection in culture which profoundly altered their response to targeted drug treatment. In 6/7 PDGFRA-mutant models, activating mutations were selected against between p5-20 in 2D and/or 3D, whilst MAPK pathway mutations in *NF1/PIK3R1* similarly diverged over 15 passages under different growth conditions, resulting in isogenic models with differential signalling, in vivo tumorigenicity, and in vitro sensitivity to multiple MEK inhibitors. In PDXs, serial xenografting reduced the time to tumour formation by up to half, with a concomitant shift in clonal architecture. Multi-region sequencing of diffusely-infiltrating tumours showed selection for alterations such as PIK3CA/NF1 at distant sites, with evidence for convergent evolution of subclonal mutations, as in human tumours. Understanding the evolutionary dynamics of targetable/predictive alterations in PDHGG model systems is key to developing new and effective therapeutic interventions in this highly heterogenous disease.

HGG-43. ABROGATION OF EXOSOME BIOGENESIS SIGNIFICANTLY AFFECTS CELL MOTILITY IN HETEROGENOUS SUB-POPULATIONS OF PAEDIATRIC-TYPE DIFFUSE HIGH-GRADE GLIOMA

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Paediatric-type diffuse High-Grade Gliomas (PDHGG) are highly heterogenous tumours comprised of distinct cell sub-populations co-existing within the same tumour mass. We have shown that primary patient-derived sub-clones, as well as optical-barcoded sub-clones, function as an interconnected network conferring an aggressive phenotype. Here, we explored the role of exosomes in mediating PDHGG inter-clonal communication. A comprehensive characterization of 7 optical-barcoded single cell-derived clones obtained from two patient-derived cell lines (one DMGH3K27-altered and one diffuse high-grade paediatric-type glioma H3WT), confirmed extensive genomic and phenotypic heterogeneity. Live single-cell tracking in 3D migration and invasion assays demonstrated the key role of the inter-clonal crosstalk in driving a more aggressive phenotype. To determine the exosome role in this crosstalk, we first characterised them in terms of size, marker expression and cargo. Moreover, we demonstrated that exosomes were actively internalized by the sub-clones. Exosomal proteomic analysis showed differential protein contents implicated in the regulation of biological processes such as focal adhesion and extracellular matrix organization. The analysis of exosomal miRNome did not show differentially expressed miRNAs between sub-clones, however, specific and distinct exosomal miRNAs were found uniquely expressed by each sub-clone. The abrogation of the exosome biogenesis by GW4869 phospholipase inhibitor did not affect sub-clones viability, but significantly inhibited their motility, when cultured individually and more prominently in co-culture condition. Analysis of the exo-miRNAs uniquely expressed by the sub-clones highlighted a set of target genes regulating cell motility/invasion/migration. These target genes were differentially expressed when sub-clones were co-cultured compared to mono-culture.

Moreover, the expression levels of these genes (e.g. CD44, PTRZ, GLI3, NTRK2) were significantly modulated upon GW4869 treatments. In conclusion, our study highlights the importance of the exosomes in the inter-clonal communication and suggests that interfering with the exosome biogenesis may be a valuable strategy to inhibit cell motility in PDHGG.

HGG-44. UNRAVELING AND TARGETING THE STEM-REGULATORY NETWORK DRIVING INVASION IN DIFFUSE HEMISPHERIC GLIOMA, H3G34-MUTANT

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Diffuse hemispheric glioma H3G34-mutant (DHG-G34) is a pediatric-type high-grade glioma affecting children and young adults. Despite surgery and radio/chemotherapy, patients have a dismal prognosis. The intratumoural heterogeneity and the high infiltrative nature of DHG-G34 cells limit the development of effective therapies. Analysing single-cell RNA sequencing data from a publicly available dataset, we identified a large and distinct sub-population of cells displaying high stem and low differentiation marker expression levels. Gene ontology analyses revealed a gene signature related to cell migration/invasion. This observation is supported by our data on in vitro 3D invasion assay and in vivo orthotopic xenograft models, showing that DHG-G34 disseminating cells are characterised by high expression level of the stem-cell marker NESTIN and low expression level of the differentiation marker GFAP. Following these findings, we developed high-throughput cell-based assays with the aim to screen a library of 1300 FDA-approved compounds and identify drugs able to induce DHG-G34 cell differentiation and inhibit their invasive phenotype. The screen, a co-immunofluorescence assay for NESTIN and GFAP, followed by dose response assays on 3D growth and 3D invasion, led to the identification of 3 FDA-approved drugs, the MEK inhibitor Cobimetinib and 2 HMG-CoA reductase inhibitors, Rosuvastatin and Pitavastatin. These 3 drugs potently induced cell differentiation (decreased Nestin and increased GFAP expression) and inhibited invasion with minimal effect on the proliferation of our DHG-G34 cell line. We are currently extending these findings to additional patient-derived DHG cell lines and we are using these drugs and different omics and imaging technologies to characterize the regulatory networks associated to DHG-G34 stemness, (de)-differentiation and invasiveness. Our work may lead to the identification of new therapeutic approaches for targeting the stem/invasive properties of these aggressive diseases.

HGG-45. CHARACTERIZATION OF SPINAL DIFFUSE MIDLINE GLIOMAS, H3 K28M-MUTANT

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Diffuse midline gliomas (DMGs) are malignant gliomas that arise in the midline structures of the central nervous system. Due to their aggressive and diffuse growth and a two-year survival rate of less than 10%, DMGs are assigned to CNS WHO grade 4. Depending on the localization, median age of patients is about 11–20 years. Genetically, most tumors are defined by a K28M-mutation in one of the highly homologous genes encoding histone protein H3. Since DMGs most frequently occur in pons and thalamus, comparatively little is known about spinal DMGs. Therefore, we histologically, molecularly, and clinically characterized spinal DMGs and analyzed, in which aspects they differ from DMGs of other localizations. Our cohort currently consists of 25 spinal DMGs and 40 pontine/thalamic reference cases. Histological, immunohistochemical and molecular analyses (DNA methylation, DNA panel sequencing) were done from FFPE tissue. Spinal DMGs were histologically very heterogeneous, both regarding different areas of single tumors as well as in comparison to other spinal and reference cases. First cluster analyses of DNA methylation data indicated a separation into three main clusters enriched for pontine, thalamic or spinal cases. The cluster enriched for spinal cases contained many tumors from elderly patients. Overall, mean age of patients with spinal DMGs was 28 years. Patients were significantly older than those with pontine DMGs. 19/20 spinal DMGs were H3-3A K28M-mutant, while one tumor had an H3-2B mutation. 4/19 (21%) spinal DMGs had mutations in FGFR1, and 6/10 (60%) in NF1. Three tumors had KRAS or BRAF mutations. In summary, first analyses suggest slight histological differences of spinal DMGs compared to DMGs of other localizations. Preliminary cluster analyses of DNA methylation data showed an enrichment of clusters for different localizations. About one third of spinal DMGs had mutations in a gene associated with the MAPK-signaling pathway.

HGG-46. INTER AND INTRA-TUMOR HETEROGENEITY OF PEDIATRIC-TYPE DIFFUSE HIGH-GRADE GLIOMA REVEALED BY HIGH-DIMENSIONAL SINGLE-CELL PROTEOMICS

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Paediatric-type diffuse high-grade gliomas (PDHGG) are aggressive brain tumors, affecting children and young adults, with no effective treatments. A main constraint to the development of effective treatment is associated with their highly heterogeneous nature. In order to further dissect their intra and inter tumor heterogeneity, we exploited the mass cytometry technology, an advanced -OMIC approach that, by using metal-tagged antibodies, allows the simultaneous measurement of more than 40 markers, at single-cell level. Here we characterized 8 primary cell lines derived from diffuse pediatric-type high-grade glioma H3-wildtype (DHGG-WT), Diffuse hemispheric glioma H3G34-mutant (DHG-G34) and Diffuse midline glioma H3K27-altered (DMG-K27) patients. The adopted antibody panel was set to recognize antigens expressed by brain and tumor cells, including H3K27M and H3.3G34R variants, and it highlighted important intra- and inter- tumor heterogeneity in the expression of the 16 considered markers. Of these, CD56, CD44, CD29 and NESTIN were more expressed in the hemispheric cell lines, while CD90 was more expressed in the pontine. Even if there was not always a concordance between CyTOF and mRNA expression data from cell lines and tumor samples (e.g. CD90 and GFAP), CyTOF data were in line with the immunohistochemistry analysis for GFAP, whose expression was significantly higher in H3.1K27 compared to H3.3K27. The UMAP analysis allowed us to identify 10 cell clusters, with very minimal overlap between hemispheric and pontine location subgroups and with a peculiar antigenic profile, whose abundance strongly varied according to the mutational subgroups. For example, while the G34 subgroup was enriched for cluster 9 (CD29/CD63/CD56/PDGRFa), the H3.1K27 was enriched for cluster 3 (H3K27M/CD90/CD63/CD56) and cluster 4 (H3K27M/CD63/CD90/CD56/GFAP). In conclusion, single-cell mass cytometry reveals a significant inter and intra-tumoral heterogeneity at protein level, dependent on the molecular alterations. This approach could contribute to the identification of new clinically relevant biomarkers for PDHGG.

HGG-47. COMPARATIVE ANALYSIS OF THE HISTONE H3 MUTANT PROTEIN INTERACTOME LANDSCAPE IN PAEDIATRIC HIGH-GRADE GLIOMAS

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There have been no significant improvements in the treatments for childhood and adolescent High-Grade Glioma (pHGG) and Diffuse Intrinsic Pontine Glioblastoma (DIPG), which have a very poor prognosis. These cancers harbour mutations affecting histone 3 (H3) proteins, 80% of DIPGs harbour histone H3.1 and H3.3 K27M somatic mutations whilst 30% of pHGGs exhibit H3.3 G34R or G34V mutations. Several studies have highlighted the epigenetic changes associated with these mutations, however their precise role in tumourigenesis is still unknown. We hypothesize that H3 mutations promote an aberrant interaction landscape and analysis of these interactome will highlight important pathophysiological consequences in these tumours. Two different affinity chromatographic proteomic analyses