dissemination reported in other case series of diagnosis of GBM-PNC and far greater than 1.1% reported for glioblastoma. In this case series, the of development of primitive neuronal component at malignant glioma recurrence was associated with radiographic appearance of a cystic mass with significant vasogenic edema as well as high rates of CSF dissemination. The clinical implication of platinum responsiveness will also be presented.

PATH-42. DIAGNOSIS AND PROGNOSTICATION OF AN ATYPICAL ADULT PRESENTATION OF DIFFUSE LEPTOMENINGEAL GLIONEURONAL TUMOR (DLGNT) BY METHYLATION PROFILING

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BACKGROUND: Diffuse leptomeningeal glioneuronal tumors (DLGNT) are rare central nervous system tumors first described in the 2016 WHO classification. A paucity of data exists on this tumor. This case describes an adult presentation of DLGNT, a classically pediatric tumor, and highlights the importance of genetic and epigenetic analyses in neuro-oncologic diagnoses. CASE: A 22-year-old male presented with shoulder paresthesias and back pain, progressing to bilateral leg weakness. Spine MRI demonstrated an enhancing intramedullary T8 lesion, prompting urgent laminectomy and resection. Frozen section suggested a glial neoplasm. Four-month follow-up MRI spine showed leptomeningeal dissemination at the C-spine and caudal thecal sac. CSF analysis revealed 110 mg/dL protein, 2 cells/mcl, and negative cytology. NCI methylation profiling demonstrated 1p deletion and KIAA1549:BRAF fusion, consistent with DLGNT, subtype 1, with MAPK pathway activation; Ki-67 index was 30%. Treatment with binimetinib was initiated. After 1 month of therapy, the patient is tolerating treatment and strength is improving. DISCUSSION: This case describes a rare presentation of a rare tumor. DLGNT, subtype 1, has a median age of diagnosis of 5 years and an indolent course. We describe an adult patient with molecular features consistent with DLGNT subtype 1, but a high proliferative index suggesting a more aggressive growth potential. In this case, methylation profiling aided in diagnosing the tumor type and guided treatment. The KIAA1549:BRAF fusion is an activating mutation in the MAPK pathway, and binimetinib was selected as a MEK inhibitor with high CNS penetration. To our knowledge, this is the first case of an adult-onset DLGNT treated with binimetinib. Based on our single patient experience, we would advocate for methylation profiling for rare CNS tumor entities.

PATH-43. HISTOLOGY OF RADIATION-INDUCED PSEUDOPROGRESSION WITH MULTIPLE FOCI IN TREATED GLIOBLASTOMA

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Radiation induced pseudoprogression (PsP) of brain tumors is defined radiologically as new or enlarging areas of non-tumor magnetic resonance imaging (MRI) contrast enhancement developing within the initial 3-6 months after completing radiation. Distinguishing PsP from genuine tumor progression is of the utmost importance in deciding the clinical course of a patient. Although it is typically diagnosed radiologically, obtaining tissue histopathology can clarify diagnostic uncertainty. However, there is no consensus on defining PsP pathologically. We present the case of a 38-year-old man with a glioblastoma, IDH-wild type, CNS WHO Grade 4 (MGMT promoter methylated) with steroid-refractory PsP, and correlate both radiologic and pathologic features. After initial treatment with a gross total resection and radiation with concurrent temozolomide, he developed a new contrast-enhancing, mass-neutral enhancing lesion consistent with PsP, which did not resolve despite 4 cycles of temozolomide and prolonged steroid treatment. The enhancing lesion was resected, and the pathological examination demonstrated areas of non-pseudopallisading necrosis, numerous macrophages, astrocytosis, hyalinization of blood vessels, and low mitotic activity in the brain parenchyma with residual infiltrative glioma. These features were consistent with radiation-treatment effect and supported a diagnosis of PsP. Over time, varying definitions for PsP include a percentage threshold of treatment effect or necrosis per sample. However, the authors recognize potential sampling bias. Therefore, we recommend including treatment-related effects coupled with the absence of tumor proliferation in the results. These features include fibrinoid necrosis, hyalinization of blood vessels, and eosinophilic coagulation necrosis. From a therapeutic standpoint, it is important to comment on the presence or absence of residual glioma cell proliferation. This case highlights a unique paring of focal histopathology with correlative sections on MRI for pseudoprogression which has not been previously presented.

PATH-44. VARIANT ALLELIC FREQUENCY OF DRIVER MUTATIONS PREDICTS SUCCESS OF GENOMIC METHYLATION CLASSIFICATION IN CNS TUMORS

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Whole genome CpG DNA methylation profiling is an extremely valuable tool in the workup of central nervous system (CNS) tumors. Reliability of such profiling depends on sufficient tumor cellularity. Many neoplastic entities have well-known driver mutations that occur in virtually 100% of tumor cells, and next-generation sequencing (NGS) assays can detect those mutations and report their relative amounts in the form of Variant Allelic Frequency (VAF). Since NGS and methylation profiling are often done on the same tumor block, we sought to determine whether driver mutation VAF affects the accuracy of methylation profiling, and whether VAF can help establish more rigorous cutoffs for quality assurance. Using NGS and Infinium Epic850K methylation arrays, we evaluated 153 CNS neoplasms representing a range of entities, including *TERT* promoter-mutant glioblastoma, *IDH*-mutant astrocytoma, *IDH*-mutant oligodendroglioma, *SHH*-driven medulloblastoma, and *CTNNB1*-driven adamantinomatous craniopharyngioma. VAFs of each driver mutation ranged between 1-60%. One hundred eleven of 153 cases (73%) had a methylation classification score ≥ 0.9, the most widely accepted cutoff for a successful result. A fit-of-mixture analysis via CutoffFinder (PMID: 23251644) suggested that the optimal VAF cutoff=31%, generating an AUC of 0.87. Ninety-six of 107 (89%) cases with a VAF of 31% or higher had a methylation classification score ≥ 0.9, whereas only 15/46 (33%) below 31% were classifiable with methylation profiling ($P< 0.0001$ by Fisher's exact test). An independent validation cohort from NYU (N=50) showed nearly identical results, with 37/50 (74%) of cases having a methylation classification score ≥ 0.9, an optimal cutoff=0.32 with an AUC=0.84, and % classification above and below the 0.32 cutoff being 30/34 (88%) and 7/16 (44%), respectively (P=0.002) These data indicate that VAF of driver mutations can serve as a useful predictor of classification success via methylation profiling, and should be taken into account when interpreting methylation profiling results.

PATH-45. INTRAEPIGLIOM - RAPID SEQUENCING-BASED DIAGNOSIS OF BRAIN TUMORS

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The intraoperative diagnosis of brain tumors remains a clinical challenge despite recent technological advances. The current clinical practice differentiates non-surgical brain tumors from those preferably treated with cytoreductive surgery employing intraoperative frozen section diagnostics. A detailed molecular diagnosis required for this classification task within the timeframe of a routine neurosurgical procedure is currently unavailable. We have analyzed a clinical cohort of several brain tumor entities using Nanopore long-read sequencing on two Oxford Nanopore Technologies sequencing platforms (MinION, PromethION). Since currently available molecular cancer classifiers such as the DKFZ methylation profiling classifier cannot be readily adapted to real-time sequencing analysis, we implemented a novel algorithm (MethyLYZR) to predict the underlying cancer type. Publicly available Illumina Infinium array data were used to train the classifier to distinguish 91 brain tumor classes. For

validation of classification accuracy, we conducted a comprehensive validation strategy. Both nanopore platforms could sequence more than 5,000 pre-selected CpG within less than 20 minutes for most of our samples. When combining an optimized library preparation protocol with the time used for sequencing the minimal number of CpGs needed for classification, we saw sample-to-answer times of less than 1 hour – in many cases within 45 minutes - from receiving a fresh biopsy to a robust cancer type prediction. Comparing actual and predicted diagnoses resulted in a favorable error rate, indicating potentially highly clinical validity. Our real-time based molecular diagnostic algorithm enables, in most cases, a reliable diagnostic call within the timeframe of a typical neuro-oncological surgery. MethyLYZR as a predictive tool may allow us to adjust the surgical strategy and deliver the prognosis to our patients right after surgery, thus allowing for as-of-yet unexplored opportunities for the intraoperative application of individualized therapeutic modalities.

PATH-46. COMPUTATIONAL HISTOPATHOLOGY INFORMED RAPID TARGETED NANOPORE SEQUENCING ENABLES AFFORDABLE NEXT DAY REPORTING OF COMPREHENSIVE MOLECULAR MARKERS FOR CNS TUMOUR DIAGNOSTICS

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BACKGROUND: Integrative brain tumour diagnostics indisputably requires comprehensive reporting of molecular markers. The 2021 WHO classification of central nervous system (CNS) tumours substantially increased the set of markers for routine evaluation, with greater significance to DNA methylation analysis in diagnostics. Limited by investment and batching, smaller labs and clinics might suffer major delays in delivering clinical decisions. To make precision diagnostics accessible, we introduce an integrated computational histopathology and adaptive nanopore sequencing workflow for next day CNS tumour diagnostics. METHODS: We used CNS-CHiP- a multitask deep transfer learning model to predict key molecular alterations and methylation classification from H&E stained CNS tumour slides. For further characterisation and subtyping, we used the predictions to formulate a custom panel for each patient. Targeted sequencing and analyses were performed using Rapid-CNS_2- a custom neurooncology nanopore sequencing pipeline for parallel copy-number, mutational and methylation analysis that is flexible in target selection with no additional library preparation and can be initiated upon receipt of frozen sections. Sequencing was performed on a portable MinION or GridION. RESULTS: We demonstrate our workflow on diagnostic samples received by the Department of Neuropathology, University Hospital Heidelberg. CNS-CHiP predicted multiple pathognomonic alterations (eg. *IDH* mutation, 7 gain/10 loss) with reasonable accuracy. This provided basic information regarding the tumor type instantly. Personalised panels enabled small target sizes, resulting in low sequencing time (up to 24h) and competitive costs. The GPU-accelerated bioinformatics pipeline reduced analysis time from > 24h to < 3h. CONCLUSIONS: Our workflow harnessing histology-based molecular predictions to instruct targeted nanopore sequencing can be set up with low initial investment and has the potential to facilitate reporting of molecular results on the next day of sample collection. CNS-CHiP combined with Rapid-CNS_2 thus aims to make CNS tumour diagnostics affordable and accessible to smaller hospitals and labs especially in low- and middle-income countries.

PATH-47. THE CHALLENGE AND THERAPEUTIC RELEVANCE OF A NON-MATCHING CLASSIFIER OUTPUT USING GENOME-WIDE DNA METHYLATION FOR CLINICAL ROUTINE

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DNA methylation-based classification of central nervous system tumours has been increasing in importance for routine clinical workups and offers novel opportunities in discriminating subtypes which could lead to a more customized therapy. However, there are still unclassifiable entities for which defining an effective therapeutic regimen is challenging. The aim of our study was to gain further insight in these challenging cases. We included 81 patients with a calibrated score below 0.9 in the classifier output, who underwent surgery for a tumour of the central nervous system (CNS). 47 patients had a different output using the classifier version v11b4 when compared to their histological diagnosis. Of these, 41 patients (87.2 %) did not have any diagnosis from the methylation classifier ("no matching methylation class"). Surgical and clinicopathological features as well as DNA input had no impact on the calibrated score. Cases with non-classifiable tumors had a significantly longer time until a decision for adjuvant therapy and these cases were presented more often in neurooncological tumor boards ($p< 0.01$). Further analyses in 23 glioblastoma patients revealed comparable results for the overall survival, but a significantly shorter progression-free survival in cases with a discrepancy between the histological and classifier diagnosis. Application of the latest classifier version v12.5 enabled classification in 67.9% of cases, resulting in re-classification with a high calibrated score (> 0.9) in 25.7% of the tumors. Taken together, our study presents unclassifiable cases and the possible clinical impact when waiting for the accurate diagnosis in these challenging cases. Even though DNA methylation profiling significantly contributes to advanced CNS tumour diagnostics, clinicians should be aware of a prolonged interval to treatment initiation, especially for highly malignant brain tumours. Therefore, we would recommend to schedule adjuvant treatment as early as possible if surgical and histological results are suspicious for this disease.

NEURO-IMAGING

NIMG-01. PREDICTING POST-STEREOTACTIC RADIOTHERAPY MAGNETIC RESONANCE IMAGE OUTCOMES OF BREAST CANCER METASTASES TO THE BRAIN

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BACKGROUND: Stereotactic radiosurgery (SRS) is a cornerstone in the management of Breast Cancer Metastases to the Brain (BCMB). While control rates are high following SRS, radiation necrosis is a rare but potentially devastating long-term toxicity. There is a clinical need for automated/semi-automated methods to assess tumor response and optimize the RT plans for local control with minimal long-term toxicity. Multiparametric MRI (mpMRI), particularly Apparent Diffusion Coefficient of water (ADC) maps, contain information that is mechanistically relatable to voxel-level tumor response to RT. We report a deep learning-based approach to predict post-SRS ADC maps, FLAIR, T2-weighted (T2W), T1-weighted unenhanced (T1W) and contrast-enhanced (T1WCE) images, from pre-SRS T1W, T1WCE, T2W and FLAIR images, ADC maps, and the delivered RT dose map. These "forward models" will enable the radiation oncologist to simulate radiologic outcomes and iteratively optimize RT plans for local control with minimal toxicity. METHODS: We trained a variant of the pix2pix Generative Adversarial Network (GAN) on MRI and RT dose map data from 18 BCMB patients treated with stereotactic radiation with confirmed controlled and locally recurrent metastases. Patients were treated with stereotactic radiation dose of 1-40 Gy between 2013-2019. RESULTS: On test data from 6 BCMB patients, the trained forward model predicted post-SRS ADC values within the Gross Tumor Volume (GTV) that were broadly in agreement with ground truth post-SRS ADC maps. In agreement with expectations, the forward model also predicts increasing post-RT ADC within the GTV with increasing simulated RT doses in the range of 1-71 Gy. We have also explored an inverse model to predict the RT dose map required to produce "prescribed" post-SRS ADC values within the GTV. CONCLUSIONS: We envision that the forward models will assist the radiation oncologist in initial RT dose plan optimization, while the inverse model may be useful for daily RT plan optimization.