Meeting Summary

69th Annual Meeting of the German Society of Neuropathology and Neuroanatomy (DGNN)

Meeting Abstracts

September 25th–27th, 2025
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Sehr geehrte Damen und Herren, liebe Kolleginnen und Kollegen,

im Namen der Deutschen Gesellschaft für Neuropathologie und Neuroanatomie heiße ich Sie herzlich willkommen zur 69. Jahrestagung unserer Fachgesellschaft, die in diesem Jahr zudem das 75-jährige Jubiläum der DGNN feiert.

Dieses besondere Jubiläum gibt uns Anlass, auf die Wurzeln und die beeindruckende Entwicklung unserer Fachgesellschaft zurückzublicken. Die Frankfurter Schule mit Persönlichkeiten wie Carl Weigert und Ludwig Edinger legte wesentliche Grundlagen für die Neurowissenschaften, deren Bedeutung bis heute kaum zu überschätzen ist.

Ein entscheidender Schritt in der Geschichte unseres Faches war der Zusammenschluss der Neuropathologen in der „Arbeitsgemeinschaft morphologisch arbeitender Neurologen und Psychiater“. Unterstützt durch den Pathologen Prof. Lauche und die Frankfurter Edinger-Stiftung fanden im Mai 1950 Vorgespräche statt, die schließlich am 7. Oktober 1950 zur Gründungsversammlung der „Vereinigung Deutscher Neuropathologen“ führten. Diese Gründung legte den Grundstein für die heutige DGNN, die seit 75 Jahren als wissenschaftliche Plattform für Austausch und Fortschritt im Bereich der neuropathologischen Forschung dient.

Besonders hervorheben möchte ich den hohen Stellenwert der Nachwuchsförderung in unserer Gesellschaft. Seit jeher ist es ein zentrales Anliegen der DGNN, junge Wissenschaftlerinnen und Wissenschaftler zu unterstützen, zu fördern und ihnen eine Plattform für ihre wissenschaftliche Entwicklung zu bieten. Nur durch die gezielte Förderung des wissenschaftlichen Nachwuchses können wir die Zukunft unseres Fachgebiets sichern und kontinuierlich neue Impulse für die Neuropathologie setzen.

Der Kongress 2025 in Frankfurt am Main bietet uns erneut die Gelegenheit, in Vorträgen und Diskussionen die neuesten wissenschaftlichen Erkenntnisse zu erörtern sowie den kollegialen Austausch zu pflegen. Besonders freue ich mich auf das breite Spektrum an Themen und den angeregten Dialog, der unsere Fachgesellschaft lebendig hält.

Mein Dank gilt allen Mitgliedern und Unterstützern, die die DGNN mit ihrem Engagement und ihrer Leidenschaft prägen und voranbringen.

Ich wünsche uns allen einen erfolgreichen und inspirierenden Kongress sowie ein bedeutungsvolles Jubiläumsjahr.

Mit herzlichen Grüßen

Karl Heinz Plate

Frankfurt am Main, im August 2025

Congress IDs

Abstract ID	P	Name
18	P28	Lea Altendorf, Hamburg
27	P29	Natalie Berghaus, Heidelberg
37	P04	Paul Kerbs, Heidelberg
38	P15	Ida Vogt, Frankfurt
39	P30	Catena Kresbach, Hamburg
43	P16	Helena Radbruch, Berlin
44	P17	Imke Metz, Göttingen
45	P24	Natalie Schoebe, Heidelberg
46	P31	Antonia Gocke, Hamburg
47	P32	Helena Bode, Hamburg
52	P07	Dorothea Münch, Frankfurt
53	P10	Antonia Neubauer, München
56	P11	Viktoria Ruf, München
57	P33	Max Braune, Leipzig
58	P34	Fuat Aras, Heidelberg
60	P12	Marina Jendrach, Berlin
62	P01	Antonia Gocke, Hamburg
63	P35	Helene Blatt, Homburg
64	P36	Felix Hinz, Heidelberg
65	P37	Ian Fichtner, Heidelberg
66	P08	Selin Kanarya, Düsseldorf
67	P38	Tanja Buhlmann, Frankfurt
68	P39	Marietta Wolter, Düsseldorf
69	P18	Raphael Raspe, Berlin
70	P13	Alberto Grassini, Tübingen

Abstract ID	P	Name
72	P40	Kimia Kafi Cheraghi, Frankfurt
73	P41	Matthias Dottermusch, Hamburg
74	P42	Laura Hruby, Düsseldorf
75	P19	Valérie Quinot, Wien
82	P43	Hannah Jessner, Münster
84	P44	Christian Thomas, Münster
85	P25	Anna Klötergens, Münster
86	P45	Rhaissa Ribeiro da Silva, Heidelberg
87	P46	Marco Münzberg, Frankfurt
88	P47	Suvendu Purkait, Düsseldorf
89	P20	Laura Schmitz-Gielsdorf, Münster
90	P21	Lidia Stork, Göttingen
91	P05	Jannik Sehring, Gießen
93	P22	Frank Heppner, Berlin
95	P48	Josefine Radke, Greifswald
96	P23	Imke Metz, Göttingen
97	P02	Anika Simonovska Serra, Wien
98	P49	Gianluca Sigismondo, Heidelberg
99	P50	Kai Schmid, Gießen
100	P26	Charlotte Brandenburg, Frankfurt
101	P06	Kiana Kafi Cheraghi, Frankfurt
102	P09	Paula Pereira de Almeida, Essen
105	P14	Maximilien Lépine, Gießen
107	P27	Willy Hube, München
108	P03	Jan Philip Kolb, Hamburg

I. Artificial Intelligence

P01

Free Neuropathol 6:17:6

Meeting Abstract

Accurate, inexpensive & fast – computational pathology for ependymoma subtyping

Yannis Schumann[1], Maximilian Middelkamp[2], Antonia Gocke[3,4], Jette Alfken[5], Sarah Becker[5], Tasja Lempertz[3], Jennifer Ahrens[3], Karoline Hack[6], Markus Glatzel[7], Claire Ertelt-Delbridge[8], Ulrich Schüller[6,7,9], Tim Salditt[5], Julia Neumann[3,7]

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Background: Ependymal tumours (EPNs) are clinically heterogeneous neoplasms and can occur in all three compartments of the central nervous system. The 2021 WHO classification defines 10 clinically distinct types of EPNs based on molecular, anatomical and immunohistochemical criteria.

Objective(s): Precise diagnoses, such as from DNA methylation analyses, are crucial for optimal therapy and patient outcome. Yet, these analyses are time consuming and expensive. We discuss multiple machine-learning methods for computational pathology of ependymomas and highlight how such approaches may act as an inexpensive and fast surrogate for molecular analysis.

Method(s): Whole-slide images of tissues with hematoxylin and eosin stain were acquired and matched with DNA methylation profiles. Interpretable machine-learning methods and multi-scale approaches were used to predict DNA-methylation types from image data. Stacking was used to incorporate basic clinical information (e.g. sex, age, location).

Result(s): We acquired a cohort of sample-matched H&E WSIs and DNA-methylation analyses of EPN (n = 597). While both CLAM (Clustering-constrained Attention Multiple Instance Learning) and HIPT (Hierarchical Image Pyramid Transformers) showed near perfect classification results when incorporating clinical variables, our results indicate that the HIPT method performed consistently better in predicting the molecular EPN types from the image data.

Conclusion(s): This study aims to leverage the prospects of computational pathology for CNS tumour diagnostics, driven by the increasing digitization of pathology workflows. Selecting the clinically relevant EPNs as use case, we demonstrate accurate predictions of molecular EPN types based on inexpensive, fast to acquire image data.

P02

Free Neuropathol 6:17:8

Meeting Abstract

Three dimensional reconstruction of neurons and connectivity patterns in the human spinal cord

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Background: Traditional histopathological staining methods are performed on specimens with a thickness of 2–5 µm, providing a two-dimensional cross-sectional view of neurons that is largely limited to the soma and proximal dendrites. A staining method that enables a three-dimensional visualization of whole neurons is the Golgi-Cox impregnation technique, which can be combined with immunofluorescent antibodies targeting specific proteins. Recent advancements in microscopy imaging and computational postprocessing techniques have further facilitated the acquisition of high-resolution images of neurons.

Objective(s): To provide a three-dimensional visualization of single neurons in the spinal cord and their connectivity patterns, as well as the dendritic spine morphology and density that are crucial for synaptic plasticity.

Question(s): To investigate differences in synaptic density in the spinal cord in the context of neurodegenerative diseases.

Method(s): In this study, human post-mortem spinal cord tissue will be used for the Golgi-Cox impregnation of spinal neurons in combination with tissue clearing and immunofluorescence staining of catecholaminergic receptors and synaptic proteins. Images will be acquired using confocal microscopy.

Result(s): Single-impregnated neurons including their soma and dendritic architecture will be segmented and subsequently reconstructed in three dimensions using AI- and deep learning-based models. The resulting images will be compiled into a comprehensive cyto- and dendroarchitectonic atlas of the lumbar spinal cord.

Conclusion(s): This staining technique can be applied to various regions of CNS and offers numerous applications in basic research, providing a deeper understanding of healthy neuronal function and potentially offering new insights into neurological disorders associated with neurodegeneration and synaptopathy.

II. Digital Pathology

P03

Free Neuropathol 6:17:9

Meeting Abstract

Reliable and instant slice-free intraoperative histology in neurooncology: updated study results with multiphoton microscopy

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Multiphoton microscopy (MPM) enables rapid, high-quality histological assessment of tissue samples without the need for paraffin or frozen sectioning. This technology can be integrated into the operating room workflow, providing results within few minutes. The aim of this study was to evaluate the diagnostic accuracy and practical applicability of MPM for intraoperative assessment of neurosurgical tumor samples. The central question was whether MPM can reliably reproduce routine histological diagnoses and thus support fast intraoperative decision-making in neurosurgery.

A total of 76 neurosurgical tumor samples were examined using MPM and compared to standard paraffin section histology. After a brief 2.5-minute staining, tissue blocks were scanned at 4 min/cm², with potential acceleration to 30 s/cm². The resulting digital images were assessed in a blinded manner by a board-certified neuropathologist with no prior MPM experience. For 43 of the 76 samples, the neuropathologist also received the suspected clinical diagnosis in addition to age, sex, and localization.

In 72 of 76 cases (94.7 %), MPM results were fully consistent with routine histology. Notably, in all 43 cases where the suspected diagnosis was provided, MPM findings matched the final diagnosis. The digital images showed excellent visualization of cell morphology and tissue architecture, closely resembling standard H&E slides. These findings highlight the reliability of MPM, even when used by neuropathologists without specific training. MPM offers a significant advance for intraoperative diagnostics, with the potential to improve patient outcomes by enabling faster and more accurate decision-making. Ongoing research will further optimize technical parameters and validate broad applicability in neuro-oncology.

P04

Free Neuropathol 6:17:10

Meeting Abstract

Longitudinal cell state screening through rapid deconvolution of intra-tumor heterogeneity in glioblastoma

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Background: Glioblastoma (GB) is characterized by intra-tumor heterogeneity (ITH), a major factor contributing to tumor progression and therapy resistance. The limited efficacy of precision therapies in GB targeting oncogenic drivers highlights the need to address ITH and its plasticity in treatment strategies. Integration of multi-omics single-cell data enabled the resolution of ITH in GB and the development of a deconvolution method which can accurately predict cell type and malignant cell state compositions from bulk methylation array data. Previous analyses revealed distinct vulnerabilities in deconvolved cell states, identifying them as potentially targetable biomarkers. We believe cell state monitoring holds significant potential for improving therapy guidance but requires rapid, scalable, and cost-effective screening methods.

Objective(s): Establishing a functional precision medicine platform modelling cell state responses in patient-derived cell lines. Enabling cell state deconvolution from sparse methylation data like liquid biopsies revolutionizing the ability to longitudinally monitor GB patients throughout their treatment.

Method(s): Methylation-based cell state deconvolution from Oxford Nanopore Technologies (ONT) sequencing data.

Result(s): We adapted cell state deconvolution to ONT sequencing data and subsequently evaluated cell state predictions in a cohort of 59 ONT sequencing samples from fresh-frozen tissue with corresponding methylation array data. Comparative analysis revealed 100% concordance in the identification of the predominant cell state.

We established the first standardized pipeline – CS_{go} (**C**ellular **S**tate resolution for **G**lioblastoma **O**ncology) – to rapidly resolve the cellular states of GB patients.

Conclusion(s): Our pipeline lays the foundation for the integration of ITH in clinical decision making and drug response monitoring.

P05

Free Neuropathol 6:17:12

Meeting Abstract

Foundation models encode technical biases: a hidden risk for multi-institutional histopathology AI

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Background: Foundation models are widely used in histopathological AI due to their capacity to encode complex morphological features. However, these models may inadvertently capture uninformative variation, including technical disparities in histological staining and digitization, potentially compromising model generalizability. This is particularly relevant for multi-institutional studies and downstream clinical applications, which require both robust cross-site performance and reliable transferability to individual laboratories.

Objective(s): Determine whether pathological foundation models encode technical biases.

Method(s): A large multi-institutional dataset of H&E-stained meningioma sections (n = 180) was compiled, with each case stained at three different institutes and digitized using three different scanners. After image registration, the feature embeddings of 100 patches per slide were encoded using seven different foundation models. To quantify the encoding of technical variables, we trained a multi-layer perceptron to classify either the scanner or the staining institute from the embeddings, keeping the other variables constant.

Result(s): Classification accuracies were evaluated across a five-fold cross-validation for each foundation model. Scanner prediction yielded very high accuracy (0.98–0.99), while institute prediction ranged from 0.73 to 0.91, depending on the foundation model used.

Conclusion(s): Foundation models encode substantial technical variation, even in controlled multi-center datasets. This raises concerns regarding interpretability and robustness, particularly when confounders correlate

with clinical labels. These biases must be considered in computational pathology workflows to avoid misleading associations and to ensure that models can be both generalized across sites and reliably deployed in individual diagnostic settings.

III. Epilepsy

Po6

Free Neuropathol 6:17:14

Meeting Abstract

Investigating neurological deficits in MEIS2-related syndrome using mESCs

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Background: *MEIS2*-related syndrome, resulting from heterozygous mutation of the transcription factor *MEIS2*, is a severe congenital disorder characterized by a triad of symptoms of cleft palate, heart malformations, and neurological deficits. The neurological side of this disorder is complex but consistently includes developmental delay with intellectual disability, severe speech and varying motor delay, autistic behavioral abnormalities, disturbed sleep patterns, and epileptic seizures.

Objective(s):

- Explore the molecular basis of neurological deficits in *MEIS2*-related syndrome
- Use an *in vitro* mESC neurogenic differentiation model to mimic early neurodevelopmental aspects

Question(s):

- Which molecular mechanisms drive the neurological symptoms of *MEIS2*-related syndrome?
- How well does mESC neurogenic differentiation recapitulate early neural development in this syndrome?

Method(s):

- mESCs with or without CRISPR-generated *Meis2* mutation subjected to neurogenic-GABAergic differentiation

- Chromatin immunoprecipitation and ChIP-seq for MEIS2 at early differentiation stages
- Transcriptome analysis and qPCR of differentiating mESCs
- Luciferase reporter assays on MEIS2-bound genomic regions
- RNA-sequencing of later neuronally differentiated mESCs

Result(s):

At early differentiation stages:

- Identification of a key mammalian brain development regulator as a direct MEIS2 target
- MEIS2-bound regions function as transcriptional enhancers

In later neuronally differentiated mESCs:

- Mutant neurons exhibit aberrant expression of specific synaptic markers
- Dysregulation of genes involved in neurotransmitter metabolism and catabolism

Conclusion(s):

Collectively, these results provide a conceptional framework for understanding the neurological deficits seen in *MEIS2*-related syndrome patients and show an unexpected link between two neurodevelopmental disorders previously considered to be unrelated.

P07

Free Neuropathol 6:17:16

Meeting Abstract

Comparison of neuroinflammatory cellular composition of tumors and peritumoral tissue in LEAT patients with and without post-surgical persistence of epilepsy

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Background: Low-grade epilepsy-associated tumors (LEAT) are primary brain tumours, usually classified as CNS WHO grade 1 lesions. LEATs are clinically associated with drug-resistant epilepsy. Early epilepsy surgery is recommended in pharmacorefractory patients. Given that surgical outcomes improve significantly when both the tumor is excised early in the disease course, and adjacent epileptogenic tissue is removed, it is assumed the perilesional tissue alterations play a vital role in the establishment of a pro-convulsive environment.

Aim: This study aims to investigate the cellular composition of LEAT and peritumoral tissue with a focus on the peritumoral zone and its potential epileptogenic role.

Methods: 27 LEAT patients were included. In each patient the cellular composition of the vital tumor and adjacent tissue were analysed. Adaptive and myeloid immune cells, astrocytes and neurons were analysed by immunohistochemistry and assessed semi-quantitatively using an image analysis software. Epigenetic alterations potentially contributing to epileptogenesis were examined in five representative tumors. The results were correlated with clinical parameters.

Results: Our preliminary results from the immunohistochemical staining show that the highest levels of lymphocytes, macrophages and microglia were found in the tumors. The peritumoral zone showed increased levels in comparison with distant tissue. Additionally, we found correlations between increased levels of immune cell infiltration and a longer duration of epilepsy.

Conclusion: The peritumoral zone constitutes a distinct microenvironment, displaying characteristic neuroinflammatory alterations that differentiate it from both the tumor and the more distant surrounding tissue. These alterations may result from seizures but could also contribute to the progression of epileptogenesis.

IV. Muscle & Nerve

Po8

Free Neuropathol 6:17:18

Meeting Abstract

Diagnosis of immune-mediated myopathies on formalin-fixed, paraffin-embedded muscle tissue samples

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Background: Immune-mediated myopathies (IMM) are the most common indication for a muscle biopsy. According to the guidelines for processing muscle biopsies, cryopreserved tissue is required for immunohistochemistry of the canonical HLA-ABC molecule of the major histocompatibility-complex (MHC) class I, the well-established biomarker of IMM.

Objective: Muscle biopsies with suspected IMM are occasionally submitted as formalin-fixed and paraffin-embedded (FFPE) specimen, in which MHC-I testing is less accurate.

Question: We tested the hypothesis that an antibody targeting the non-canonical MHC-I molecule HLA-E may overcome this limitation and can be used on cryo- and FFPE tissue.

Method: We examined HLA-E expression on fifty frozen muscle biopsies, clinically and histologically diagnosed as IMM, and ten frozen samples with morphological hallmarks of neurogenic muscular atrophy without signs of inflammation. Subsequently, we tested the same HLA-E antibody on twenty FFPE muscle biopsies with confirmed inflammation in which MHC-I expression could not be determined up to now, as well as in ten FFPE samples without inflammation as controls.

Result: Immunoreaction of HLA-E antibody successfully detected sarcolemmal and/or sarcoplasmatic upregulation associated with inflammation. This was true for both frozen and FFPE samples in specimens in which the diagnosis of IMM has been established based on frozen samples. In contrast, in samples with neurogenic muscular atrophy both canonical MHC-I and HLA-E expression were absent.

Conclusion: Immunostaining for HLA-E enables the diagnosis of IMM using FFPE muscle samples when frozen tissue is unavailable, offering a valuable diagnostic alternative in cases in which tissue handling did not adhere to the guidelines.

V. Neurodegeneration

P09

Free Neuropathol 6:17:19

Meeting Abstract

The interaction between Kallikrein-8 and its inhibitory network affects Alzheimer's disease

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Background: Increased Kallikrein-8 (KLK8) level seems to contribute to Alzheimer's disease (AD) pathology, but it remains unknown whether a disruption of its inhibitory network is involved. Serpin A3, serpin B6, alpha-2-macroglobulin (α2M), and the acetylcholine (ACh)- producing protein PEBP1 are the putative inhibitors of KLK8.

Objective: To examine KLK8↔inhibitor interactions and their functional consequence in AD pathology.

Methods: We combined in silico modeling and enzymatic assays to assess binding and inhibition. Complex formation was validated by co-immunoprecipitation, western blot and mass spectrometry, and was quantified in the AD pathology affected human and murine hippocampus. Cellular models (SH-SY5Y, primary glia) tested KLK8 inhibition effects on Aβ phagocytosis, neurite outgrowth, and ACh production.

Results: A Protein-protein complexation between KLK8 and its putative inhibitors was predicted by AlphaFold Artificial Intelligence (AI). All four inhibitors bound and inhibited KLK8 in vitro, and KLK8 inhibition restored neurite outgrowth and boosted Aβ clearance in cellular models. KLK8 complexes with serpin A3, serpin B6 and α2M were significantly reduced in human (but not murine) hippocampus when affected by AD pathology. The reduced ACh levels upon KLK8 exposure in SH-SY5Y cells were countered when KLK8 was pre-incubated with PEBP1.

Conclusion: Our results indicate that reduced KLK8 complexation with its inhibitors α2M Serpin A6 and B6 may contribute to higher KLK8 activity as seen in AD brain, while increased KLK8↔PEBP1 interaction might explain the compromised cholinergic signaling in AD brain. These results underscore the therapeutic potential of targeting KLK8's regulatory network in AD.

P10

Free Neuropathol 6:17:20

Meeting Abstract

Associations between age, sex and ApoE with amyloid-beta, tau and α-synuclein loads in Alzheimer's disease

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Background: Age, ApoE-genotype and sex influence the risk for Alzheimer's disease (AD) and Lewy body dementia. The relationship with mixed pathologies requires further elucidation.

Objectives: We aim to assess the association between age, ApoE4, and sex with α-synuclein (α-syn), amyloid-beta (Aβ) and tau load in AD and how these factors are distributed with variable α-syn deposit distributions.

Methods: 72 advanced AD cases of the Neurobiobank Munich were included. α-syn, Aβ, and tau deposits were automatically quantified with random forest pixel classifiers on diaminobenzidine stainings for α-syn42, 4G8, and AT8 in up to 28 brain regions per case. Associations between deposit loads and age at death, sex, and ApoE4 were analyzed with multiple linear regression models. Additionally, age, sex, and ApoE4 carriage were compared between α-syn co-pathology distributions.

Results: While female cases showed a significantly higher cortical and hippocampal Aβ load, males presented with a higher hippocampal and amygdala-entorhinal tau load. Patients with a younger age at death showed focally higher Aβ and tau loads. ApoE4 carriers had higher cortical Aβ loads and an increased hippocampal α-syn load. Although not significantly, there was a trend towards more female cases and more ApoE4 carriers presenting with α-syn co-pathology. AD cases with cortically disseminated α-syn deposits tended to have a lower age at death.

Conclusion: Despite a limited cohort selection, age, sex, and ApoE-genotype showed associations with Aβ and tau load, and α-syn co-pathology in AD. Therefore, these factors should be considered in patient stratification for therapeutic trials.

P11

Free Neuropathol 6:17:21

Meeting Abstract

White matter granular astrocytic tau inclusions are a highly consistent feature in multiple system atrophy

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Background: Multiple System Atrophy (MSA) is a fatal neurodegenerative disorder characterized by alpha-Synuclein aggregates in oligodendrocytes (*glial cytoplasmic inclusions*; GCIs). Furthermore, small granular tau inclusions in white matter have occasionally been described, however, these were controversially discussed.

Aim: Based on our own observations and our snRNA-Seq data showing significant upregulation of *MAPT* in astrocytes from MSA patients, we aimed to characterize their presence more systematically.

Methods: Immunohistochemistry and immunofluorescence were applied to archived FFPE tissue from the Neurobiobank Munich.

Results: We confirmed the presence of granular tau inclusions, which differ from previously described tau pathologies, and excluded tissue processing or staining artifacts as their cause. The inclusions, also positive for RD3, RD4, pTau-thr231 and pTau-ser396, were closely associated with astrocytes but not with oligodendrocytes or microglia. Importantly, there was no apparent colocalization of tau inclusions and GCIs. In parallel, we reviewed archival MSA cases along with cases with other neurodegenerative diseases and healthy controls. While we detected granular astrocytic tau inclusions (GATIs), though to different extents, in all MSA cases examined (n = 45), they were absent in other neurodegenerative diseases or healthy controls. GATIs were distributed throughout the white matter of the entire brain and spinal cord. The regional distribution closely mirrored that of GCIs and we also found a positive correlation of the burden of GCIs and GATIs.

Conclusions: GATIs appear to represent a highly consistent MSA-specific tau pathology, distinct from previously known tau pathologies, with their distribution strongly paralleling alpha-Synuclein pathology. Their pathological significance, however, remains to be clarified.

P12

Free Neuropathol 6:17:22

Meeting Abstract

Impact of the gut microbiome on microglia and Alzheimer's disease in different mouse models

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Background: The gut microbiome plays a crucial role in shaping the immune response, which is closely linked to the development and progression of Alzheimer's disease (AD). Standard laboratory mice, known as SPF mice, have a limited and less diverse microbiome than mice living in the wild or humans, resulting in an immune system that may not fully reflect the immune response of adult human. This difference may potentially reduce their translational relevance.

Objective(s): Wildling mice, are laboratory mice that have a natural microbiome, comparable to a mouse in the wild. We are part of the framework of the "Charité 3R Wildling mice in Health and Disease" consortium, where the wildling mouse is validated in different pathological settings.

Question(s): Do Wildling mice provide a more translationally relevant model for studying Alzheimer's disease?

Method(s): AD pathology was assessed in SPF and Wildling mice using electrochemiluminescence assays, mass spectrometry, and immunohistochemistry. Gut microbiota composition was analysed, and immune profiling was performed by flow cytometry.

Result(s): We examined wildtype Wildling mice and transgenic APPPS1.Wildling mice in direct comparison to their SPF counterparts. Wildling mice displayed a significantly different gut microbiome composition and immune cell population. Notably, APPPS1.Wildling mice showed signs of AD-related pathology already at early disease stages, earlier and more pronounced than in SPF mice.

Conclusion(s): Our results suggest that wild-type mice more accurately reflect important aspects of human AD pathology, particularly regarding gut-brain interactions.

P13

Free Neuropathol 6:17:23

Meeting Abstract

First report of clinico-pathological features associated with TARDBP p.M311V mutation

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TDP-43 is the aggregating disease protein in most patients with ALS and in ~50 % of patients with FTD. Mutations in the encoding gene *TARDBP* are usually associated with clinical ALS. Few clinical reports describe combined ALS and FTD or pure FTD with *TARDBP* mutations; however, detailed clinico-pathological reports from *TARDBP* mutation carriers are rare.

We report a 55-year-old man with a *TARDBP* p.M311V mutation. Symptoms started at age 54 with impaired naming and single-word comprehension, fulfilling diagnostic criteria for semantic variant of primary progressive aphasia (svPPa). About a year later symptoms were accompanied by behavioral alterations and signs of motor neuron disease. The patient deceased ~18 months after disease onset. Neuropathological examination revealed a severe atrophy of the temporal lobe and amygdala with marked asymmetry (left > right). Degeneration of the primary motor system was moderate. Pronounced TDP-43 pathology was observed in these regions predominantly as compact neuronal cytoplasmic inclusions; however, their morphology and laminar distribution pattern did not correspond to any of the known FTLD-TDP subtypes.

The observed svPPA due to predominant temporal atrophy in this p.M311V patient is comparable to reports of two patients with a p.I383V mutation. It remains to be seen, if this is a consistent feature of the p.M311V mutation; however, our findings imply that some *TARDBP* mutations are associated with a higher toxicity to neurons in the temporal lobe than to motor neurons. Identification of factors modifying the consequences of *TARDBP* mutations will be crucial for better understanding of the pathogenesis underlying TDP-43 proteinopathies.

VI. Neuroinflammation

P14

Free Neuropathol 6:17:24

Meeting Abstract

Borna disease virus 1 infection in organotypic hippocampal slide cultures from adult rats

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Background: Borna Disease Virus 1 (BoDV 1) is a zoonotic and neurotropic virus that causes fatal non-suppurative encephalitis in humans, horses, sheep, and alpacas. The white-toothed shrew (*Crocidura leucodon*) has been identified as a natural reservoir host, harboring a persistent infection without developing neurological impairments. For infection studies, the adult rat serves as a suitable model for studying inflammation in dead-end hosts.

Method(s): Viability of organotypic hippocampal slice cultures of adult rats was analyzed by LVE/DEAD immunofluoresence staining, LDH assay, PCR of housekeeping genes and morphologic integrity. In a second step, viral spread, infection patterns, and the local innate immune response were analyzed using immunofluorescence and qPCR to assess viral load.

Result(s): OHCs were susceptible to infection while maintaining tissue integrity for up to 28 days in culture. Viral distribution was uniform across hippocampal regions, but viral load decreased between days 3 and 7 post-infection (p.i.) before increasing between days 7 and 21 p.i.

Conclusion(s): These results demonstrate that OHCs provide a valuable model for studying viral persistence, distribution, and load in adult rats.

P15

Free Neuropathol 6:17:25

Meeting Abstract

Immune profile and clinical characteristics of Central Nervous System Graft-versus-Host-Disease

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Background: Central Nervous System Graft-versus-Host Disease (CNS-GVHD) is a rare complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT). Early diagnosis and treatment are essential to halt disease progression. Data on risk factors, disease mechanisms and treatment is scarce. While clinical criteria have been suggested, histopathological criteria are lacking.

Objective(s): This project aims to assess frequency, clinical features and histopathological findings to improve our understanding of its immune microenvironment.

Question(s):

1. Is CNS-GVHD a clinically significant complication in allo-HSCT patients?
2. What insights do CNS-GVHD biopsies reveal?
3. Can CNS-GVHD diagnosis criteria be improved?

Method(s): 1436 patients treated with HSCT at the University Hospital Frankfurt between 01.01.2014 and 31.07.2024 were retrospectively examined for CNS-GVHD, risk factors and outcomes. In an additional multicenter CNS-GVHD cohort of 7 biopsied patients, intensity, localization, cellular composition of inflammation, necrosis and gliosis were assessed.

Result(s): Of 1436 HSCT patients, 786 received their first allo-HSCT. Among those, 5 patients were diagnosed with CNS-GVHD, corresponding to a prevalence of 0.6 %. CNS-GVHD manifested shortly after discontinuation of immunosuppression in all patients. Under high-dose glucocorticoid therapy, clinical response was varying. Histologically, CNS-GVHD displayed perivascular and parenchymal infiltrates, predominantly consisting of CD8-positive T-lymphocytes. We identified two inflammation patterns: one adaptive-immune-response pattern dominated by lymphocytic infiltration (4 patients) and one innate-immune-response pattern dominated by macrophage infiltration and phagocytosis (3 patients).

Conclusion(s): CNS-GvHD is uncommon but clinically significant. Its manifestation shortly after discontinuation of immunosuppression supports a GVHD-related pathophysiology. Histopathological findings suggest distinct immune response patterns that may reflect different stages of CNS-GVHD.

P16

Free Neuropathol 6:17:27

Meeting Abstract

HPgV-1 is infecting glial cells in immunosuppressed patients

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Background: Pegivirus hominis (HPgV-1) is a single-stranded RNA flavivirus previously considered non-pathogenic in humans, although it has been reported in association with leukoencephalitis and myelitis in e.g. the context of HIV.

Objective(s): We want to investigate the neuropathological manifestation of Pegivirus-associated encephalomyelitis (PAEM).

Question: Is PAEM a distinct encephalomyelitis in immunocompromised patients?

Method(s): Here, we report a neuropathological work up of two chronically immunosuppressed patients presenting with an encephalomyelitis associated with HPgV-1 detection in the central nervous system (CNS).

Result(s): Autopsies revealed infiltration by macrophages and T cells, upregulation of HLA-DR, tissue destruction — findings compatible with viral encephalomyelitis in areas where we could detect HPgV-1 RNA by PCR and In-situ hybridization (ISH). PgV-1 RNA viral loads were significantly higher in central and peripheral nervous system compared to peripheral organs, with the highest viral loads in the optic nerves and cervical spinal cord, corresponding to the affected clinical and radiologic regions. ISH showed viral RNA mainly in glial cells of the white matter. Analysis of complete HPgV-1 genome sequences obtained from tissue samples revealed single nucleotide polymorphisms, amino acid substitutions, and deletions within the CNS.

Conclusion(s): PAEM is a distinct and severe form of encephalomyelitis in chronically immunosuppressed patients, predominantly affecting the optic tracts as well as the motor and sensory pathways of the brain and spinal cord. The detection of viral RNA in glial cells, genetically distinct viral populations and evidence of independent viral replication in the CNS supports a causative role of HPgV-1 in PAEM.

P17

Free Neuropathol 6:17:28

Meeting Abstract

T cell infiltration patterns and clinical correlates in natalizumab-related PML lesions

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Background: Progressive multifocal leukoencephalopathy (PML) is an opportunistic viral demyelinating brain disease caused by the JC virus that may occur during natalizumab treatment for multiple sclerosis. Tissue resident memory (T_{RM}) CD8+ T cells are long-lived stationary immune cells that provide key frontline defense against viral reactivation. CD4+ and CD8+ T cell populations are reported to be essential for antiviral immunity in PML.

Method(s): We investigated T cell infiltration patterns (T_{RM} CD8+ T cells, CD4+ T cells, CD8+ T cells), viral clearance, and clinical outcomes in natalizumab-associated PML, and compared them with classic PML lesions (< 500 T cells/mm²) and inflammatory PML lesions (> 500 T cells/mm²) from patients without prior natalizumab exposure. Clinical outcomes were assessed using the Expanded Disability Status Scale (EDSS) and modified Rankin Scale (mRS).

Result(s): PML lesions following natalizumab treatment showed high T cell infiltration including T_{RM} CD8+ T cells (164.2 cells/mm²), comparable to inflammatory PML lesions without natalizumab pretreatment. Elevated T cell numbers persisted until 234 days after the last natalizumab infusion. Approximately four months after natalizumab withdrawal, profound reduction of virally infected cells was evident (> 120 days: 1.8 cells/mm²). Negative correlation between CD8+ cytotoxic T cells and T_{RM} CD8+ T cells with clinical disability scores was observed (EDSS: p = 0.05, p = 0.03; mRS: p = 0.004, p = 0.03).

Conclusion(s): After natalizumab withdrawal in PML, immune system reconstitution occurs with profound viral clearance at approximately four months. CD8+ cytotoxic T cells, including T_{RM} CD8+ T cells, seem to play crucial roles in viral control and are associated with improved clinical outcomes.

P18

Free Neuropathol 6:17:29

Meeting Abstract

Multiplexed viral detection in brain tissue – methodological challenges and opportunities

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Background: Following initial claims for viral detection in CNS tissue during the SARS-CoV-2 pandemic, subsequent studies revealed reduced specificity of immunohistological stains and common misidentification of cellular structures as viral in studies using electron microscopy.

Objective(s): The resulting scientific debate on viral presence in the CNS highlights the utmost importance of robust methods for specific detection of viral products.

Method(s): In a multicenter approach, we generated tissue microarrays containing 380 thalamic and cerebellar samples from donors deceased of COVID-19 (n = 134) and age-matched prepandemic controls (n = 71). SARS-CoV-2 transcriptional abundance and interferon-related gene expression were analyzed using 10x Xenium probe-based spatial transcriptomics, with pulmonary tissue of COVID-19 donors serving as control for active infection and three HSV1 probes serving as control for neurotropism. qPCR and immunohistochemistry on a subset of samples served as orthogonal validation.

Result(s): Pulmonary controls contained SARS-CoV-2 RNA transcripts, and brain samples from one systemically HSV1-infected but not previously diagnosed donor showed cells positive for all three HSV1 probes. Among all other samples, significant but weak signal for both SARS-CoV-2 and HSV1 probes was restricted to granule cell areas of few PCR-negative cerebellar samples and correlated closely with negative control background.

Interferon-related gene expression in early COVID-19 brain samples was elevated in comparison to controls and late COVID-19.

Conclusion(s): Our results point to an acute bystander activation of the CNS during systemic COVID-19 infection, rather than true neurotropism of SARS-CoV-2. They highlight the importance of using orthogonal methods for viral detection.

P19

Free Neuropathol 6:17:31

Meeting Abstract

Neuropathological characteristics of Anti-GABA-B-Receptor-Encephalitis

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Background: Antibodies (AB) targeting the γ-aminobutyric acid type B receptor ($GABA_BR$) lead to symptoms associated with limbic encephalitis, including seizures, memory loss and confusion. 50 % of patients have underlying small-cell lung cancer (SCLC). Recently, AB directed against the potassium channel tetramerization domain-containing (KCTD) protein 16, accessory to the $GABA_BR$-complex, have been associated with paraneoplastic anti-$GABA_BR$-encephalitis with SCLC.

Objective(s): To characterize the type and topographic distribution of cellular and humoral inflammatory response in post-mortem brains and to correlate the findings with clinical data.

Method(s): Histological and immunohistochemical evaluation of 4 archival brain autopsies from patients with anti-$GABA_BR$-encephalitis. Determination of serum-anti-KCTD16-AB-status by CBA.

Result(s): Neurological symptoms of the 4 patients (4 males, mean age of 74a) included epileptic seizures (n = 2), dysarthria (n = 1), dysphagia (n = 1), and vertigo (n = 1). 1 patient had SCLC. On histology, mild to moderate CD8+ T-cell-dominated parenchymal and perivascular inflammation in the patient with positive anti-KCTD16-AB-status, and a smaller fraction of CD79+ B-cells/plasma cells was observed in amygdala, hippocampus, and cingulate gyrus compatible with predominantly limbic encephalitis, followed by inflammation in brainstem and basal ganglia in all patients. Microglia profile was consistently proinflammatory (HLA-DR+, p22phox+). One patient showed

bilateral hippocampal sclerosis, while unilateral hippocampal sclerosis was identified in 2/4 patients. Two patients showed combined neurodegenerative proteinopathies.

Conclusion(s): Our results indicate a T-cell-inflammatory component in addition to an AB-mediated pathophysiology in anti-$GABA_BR$-encephalitis, which may be more pronounced in the paraneoplastic form associated with SCLC and anti-KCTD16-AB.

P20

Free Neuropathol 6:17:33

Meeting Abstract

Impact of myelin phagocytosis on human blood-derived macrophages and microglia and its effect on human oligodendrocytes

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Background: Myelin-debris clearance by myeloid cells in multiple sclerosis (MS)-lesions is essential for remyelination, as myelin-debris itself can impede this process. However, the effects of myelin-uptake on macrophages remain controversial, with studies reporting both pro-inflammatory and disease-resolving phenotypes that could either hinder or promote remyelination. Notably, human-based data supporting the latter hypothesis and investigating subsequent effects on human oligodendrocytes are lacking.

Objective(s): Here we investigate phenotypic changes in myeloid-cells due to myelin-uptake at different stages of myelin-processing and their impact on human oligodendrocyte-differentiation and (re-)myelination.

Method(s): We analysed oligodendrocyte numbers and remyelination in 28 lesions from 25 MS-patients, comparing lesions with and without lipid-droplet (LD)-forming myeloid cells. Myeloid-cells from controls and MS-patients were exposed to control or MS-derived myelin for various periods. Inflammatory profiles were assessed, and conditioned media were applied to iPSC-derived oligodendrocytes (hiOL) to evaluate effects on differentiation.

Result(s): No correlation was found between LD-forming macrophages and oligodendrocyte numbers or remyelination in MS-lesions. *In vitro*, myelin-uptake led to a pro- to anti-inflammatory shift in healthy myeloid-cells, yet hiOL differentiation was inhibited at both stages. Unexpectedly, in MS-macrophages, control myelin had minimal effect, while MS-myelin induced polarization and again inhibited hiOL-differentiation.

Conclusion(s): Although myelin-uptake promotes an anti-inflammatory shift in myeloid-cells, this did not support oligodendrocyte-differentiation. Furthermore, LD-formation did not associate with remyelination in MS-lesions. Concluding: contrary to published animal studies, our findings, using human oligodendrocyte lineage cells, show no evidence for a role of LD-formation in macrophages on remyelination failure and no evidence that an anti-inflammatory phenotype promotes oligodendrocyte-differentiation.

P21

Free Neuropathol 6:17:34

Meeting Abstract

Remyelination in multiple sclerosis depends on the mechanism of lesion development

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Immunopathological patterns are histological signatures reflecting the heterogeneity of multiple sclerosis (MS), encompassing clinical symptoms and treatment response. Patterns suggest different mechanisms of lesion development: pattern I and II with immune-mediated demyelination, and pattern III reflecting primary oligodendrocyte damage.

We hypothesized that these mechanisms also influence remyelination in MS. Using the BCAS1 marker for remyelinating oligodendrocytes, we analyzed the number and morphological characteristics of BCAS1+ cells across the three patterns. Additionally, we analyzed oligodendrocyte precursor cells (strong Olig2+) and mature NogoA+ cells.

For oligodendrocyte precursor cells, no significant difference was observed between patterns ($p = 0.4$), but mature NogoA+ oligodendrocytes were slightly higher in pattern III. The number of BCAS1+ cells exhibited considerable variability among patients, with some showing low (median: 4.5 cells/mm²), moderate (median: 15.5 cells/mm²), or high (median: 44.1 cells/mm²) numbers. No significant differences were found between the immunopathological patterns for BCAS1+ cells ($p = 0.9$). To assess active myelination, we used double immunofluorescence with myelin-associated glycoprotein (MAG). The ratio of BCAS1+MAG+ to all BCAS1+ cells was similar across patterns, but pattern III was characterized by a significantly higher number of ramified BCAS1+MAG+ oligodendrocytes (median: pattern I: 0.2, pattern II: 0.2, pattern III: 0.5 cells; $p = 0.04$).

In conclusion, these findings indicate that lesions of pattern III, despite being characterized by primary oligodendrocyte dystrophy, paradoxically demonstrate a significantly higher number of mature oligodendrocytes and ramified, active myelinating BCAS1+ cells in areas of tissue response, potentially reflecting an enhanced reactive regenerative capacity and superior remyelination potential compared to other lesional patterns.

P22

Free Neuropathol 6:17:35

Meeting Abstract

IL-12 drives neuroinflammation-linked lipid dysregulation in a mouse model of Alzheimer's disease

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Background: Neuroinflammation is central to Alzheimer's Disease (AD) pathology. Microglia-derived interleukin (IL)-12 promotes inflammation and disease progression in AD patients and APPPS1 mice, which recapitulate the first stages of AD, with amyloid beta (Aβ) accumulation, neuroinflammation, and myelin disruption.

Objective(s): Lipid metabolism seems to be altered in AD, but the interplay with neuroinflammation is yet unclear. Therefore, we aimed to characterize lipidomic alterations and metabolic changes in key brain cells during AD progression in the APPPS1 mouse model and clarify IL-12's role in these processes.

Question(s):

1. How is the lipidomic profile, especially sphingolipids and myelin-related lipids, affected at different disease stages?
2. How do Aβ and myelin pathology impact metabolic function in diverse cell types?
3. Does IL-12 play a role in modulating these pathological features?

Method(s): We performed sphingolipid-targeted lipidomics by LC-MS on gray and white matter from wild type, APPPS1, and APPPS1.*Il12b*$^{-/-}$ mice at 4 and 8 months and used immunohistochemistry to evaluate lipid metabolism-related proteins.

Result(s): 4-month-old APPPS1 mice show distinct lipid profile changes vs. wild type mice that diminish with disease progression. Parvalbumin-positive (PV^{+}) interneurons display reduced LAMP^{+} lysosomes, signaling impaired metabolism, while PLIN3^{+} lipid droplet levels remain stable. Microglia accumulate more, smaller BODIPY^{+} lipid droplets in APPPS1, whereas wild type microglia have fewer, larger droplets. *Il12b* deletion partially restores these phenotypes.

Conclusion(s): Our findings support IL-12's role in neuroinflammation-driven lipid metabolism disruption and support IL-12 inhibition as a potential AD therapy.

P23

Free Neuropathol 6:17:36

Meeting Abstract

Development and validation of a multiplex microarray for pathogen detection in paraffin-embedded CNS tissue

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Background: Inflammatory CNS diseases can be caused by different pathogens, including viruses, bacteria, fungi, and protozoa. Differential diagnoses include autoimmune diseases that require different therapeutic approaches. Histopathology may not always detect pathogens, and specific antibodies or stainings are not available for all pathogens.

Objective(s): To develop a comprehensive, practical test to detect pathogens in paraffin-embedded CNS tissue.

Method(s): A new CNS multiplex microarray chip based on the Vision*Array*® system is developed in collaboration between ZytoVision GmbH (a Zytomics company), and the Institute of Neuropathology, University Medical Center Göttingen, encompassing the most important pathogens for CNS infections in Europe.

Result(s): High sensitivity was found for toxoplasmosis and progressive multifocal leukoencephalopathy (9/9 and 13/13 cases positive, respectively). Four of five cases with inflammatory necrotic lesions in immunosuppressed patients suspicious for toxoplasmosis but negative on immunohistochemistry were also positive. For progressive multifocal leukoencephalopathy, 3/4 cases suspicious for PML but negative on conventional testing were positive. No false positive results were found. Tests for other pathogens still require optimization.

Conclusion(s): This CNS microarray shows promise for detecting certain pathogens with high sensitivity and specificity, providing clear added value compared to histopathology alone.

VII. Single-cell Technologies

P24

Free Neuropathol 6:17:37

Meeting Abstract

Investigation of intratumour heterogeneity in primary and recurrent glioblastoma via single cell whole exome sequencing

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Background: Glioblastoma (GBM) are aggressive brain tumours with high rates of recurrence, likely driven by a high level of intratumour heterogeneity. Yet, standard DNA sequencing methods do not allow for single cell resolution.

Objective(s): To characterize subclonal dynamics in the transition from primary to recurrent tumour and uncover potential mechanisms of therapy resistance.

Question(s): Which subclones persist, evolve or emerge under therapeutic pressure and through which molecular mechanisms do they gain this advantage?

Method(s): Primary and recurrent GBM samples from two patients who underwent standard of care treatment were analysed. Fresh frozen tumour tissue was dissociated, and single nuclei were sorted into 384-well plates for primary template-directed amplification (PTA), a single-cell whole genome amplification method enabling single nuclei genome (snWGS) and exome sequencing (snWES) while ensuring uniform genome coverage and low amplification bias.

Result(s): SnWGS from pair 1 revealed canonical chr7 gain and chr10 loss, with additional alterations such as chr3 loss in the recurrent tumour. Pair 2 showed a shift from partial to a full 7/10 signature, with other unique

changes between states. For example, the primary tumour harboured a chr22 loss that was not present in the recurrent sample which, in turn, presented a chr11q loss.

Conclusion(s): PTA snWGS CNV profiling detects subtle genomic changes: Overall, the primary tumours showed more subclonal alterations than the recurrent ones, suggesting an evolutionary selection for certain treatment resistant subclones after recurrence. Additional snWES will provide more insight into treatment-resistant tumour clones and their mutations and mutational signatures.

P25

Free Neuropathol 6:17:39

Meeting Abstract

Exploring inter- and intratumoral heterogeneity of choroid plexus tumors on a single cell level

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Background: Choroid plexus tumors (CPTs) are rare intraventricular neoplasms arising from choroid plexus (ChP) epithelial cells, predominantly affecting children. They include three histological subtypes: choroid plexus papilloma (CPP), atypical CPP (aCPP), and choroid plexus carcinoma (CPC), and cluster into three epigenetic groups: pediatric A, pediatric B, and adult.

Objectives: We aimed to elucidate the cellular composition and molecular underpinnings of CPTs across histological and epigenetic subtypes.

Methods: Single-cell transcriptomics was applied to 43 CPTs spanning all histological and epigenetic subtypes, alongside 12 normal ChP samples. Spatial transcriptomics was conducted on 15 tumors and 2 non-neoplastic ChP samples. Integration with fetal, pediatric, and adult ChP tissue enabled the creation of a comprehensive single-cell atlas of human ChP cells, illuminating developmental and tumor-related transcriptional programs.

Results: Our analyses revealed distinct molecular profiles by subtype and treatment status. Notably, pediatric B papillomas resembled carcinomas more than other papillomas, sharing signaling pathway enrichment, loss of motile cilia, and similar CNV patterns. Spatial transcriptomics uncovered organized myeloid populations with subtype-specific gene signatures, implicating them in tumor-stroma interactions. Notably, both CPC-associated myeloid and tumor cells exhibited enhanced interferon signaling, which decreased following treatment. This reduction aligns with our observation that treated CPC cells cease cycling and regain ciliary function, suggesting a profound molecular reprogramming.

Conclusion: This study reveals previously unexplored aspects of CPT biology, providing novel insights into the cellular and spatial heterogeneity of CPT subtypes, thereby paving the way toward more personalized and effective treatment strategies for patients with CPT.

VIII. Tumor

P26

Free Neuropathol 6:17:40

Meeting Abstract

Analysis of histological, molecular and preanalytic features of unclassifiable CNS specimens: comparison of the Heidelberg v12.8E, Bethesda v3 methylation-based brain tumor classifiers and the Hetairos H&E histology-based classifier

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DNA methylation profiling has become an important tool in neuropathological diagnostics, often enabling precise WHO classification of CNS tumors. However, a subset of cases remains unclassifiable using current methylation-based brain tumor classifiers. We retrospectively analyzed a cohort of 1,853 CNS tissue samples diagnosed between 2017 and 2024 using genome-wide DNA methylation profiling and identified a cohort of 393 cases (21 %) unclassifiable by the Heidelberg Brain Tumor Classifier v12.8E. For a representative subset of 252 cases, we compared the classification results of the Bethesda Classifier v3 (Bv3) and Hetairos, a novel classifier predicting methylation classes from digital H&E-stained slides, and examined preanalytic, histological, and molecular features. Among 252 cases that were unclassifiable by v12.8E, 107/252 cases (42 %) were correctly classified using the Bv3 (unclassifiable cases: 118/252 - 47 %; alternative diagnostic suggestion: in 27/252 cases - 11 %). 93/252 cases (37 %) were correctly classified by Hetairos (unclassifiable cases: 145/252 - 58 %; alternative diagnostic suggestion: in 14/252 cases - 6 %). The Bv3 correctly identified 74/112 glioblastomas,

IDH-wildtype (GBM; 66 %), including 76 % histological (61/80) and 41 % molecular (13/32) GBM. Hetairos identified 78/112 GBM (70 %), including 75 % of the histological (60/80) and 56 % of the molecular GBM (18/32). In both cases, the RF_purify-estimated tumor cell content of correctly classified GBM samples was significantly lower compared to 458 GBM cases correctly classified by v12.8E ($p = 0.01086$ and $p = 0.01966$, respectively). These results highlight the complementary value of Bv3 and Hetairos in reliably classifying diagnostically challenging cases, especially in samples with low tumor cell content.

P27

Free Neuropathol 6:17:42

Meeting Abstract

Immunothrombosis and endothelial priming in early brain metastasis

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Background: The incidence of brain metastases is rising among patients with systemic malignancies, yet preventive strategies targeting the earliest phases of metastatic seeding into the CNS remain lacking. Emerging evidence points to the critical role of vascular and thromboinflammatory responses during the initial arrest of circulating tumor cells in the brain microvasculature—a process that mirrors key aspects of ischemic stroke.

Methods: Using syngeneic mouse models, we injected tumor cells into the internal carotid artery to induce brain metastases and analyzed early host responses via high-resolution spatial transcriptomics. Transcriptional profiling was performed at days 1 and 4 post-injection, focusing on the perivascular microenvironment surrounding arrested CTCs. Operating under the hypothesis that early thrombotic events are critical for brain metastasis seeding, we investigated the use of Rivaroxaban as a preventive anticoagulant therapy.

Results: Endothelial cells exhibited the most pronounced early transcriptional response, with upregulation of vascular remodeling genes. This gene expression was spatially restricted to areas in direct proximity to tumor cells. Glial cells near these sites expressed angiogenic factors as well, indicating a supportive role of the glial compartment in pre-metastatic niche formation. Notably, we observed lymphocyte-rich thrombi containing intravascular tumor cells. Treatment with the anticoagulant Rivaroxaban significantly reduced tumor clot formation, and the development of parenchymal brain metastases.

Conclusion: Our findings identify endothelial activation and thromboinflammatory signaling as key early events in brain metastasis formation, paralleling mechanisms seen in ischemic stroke. These insights suggest that combinatory antithrombotic therapies may offer a viable preventive strategy against metastatic brain colonization.

P28

Free Neuropathol 6:17:43

Meeting Abstract

Molecular and histological analyses of AT/RT-TYR suggest the choroid plexus of the fourth ventricle as cellular origin

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Atypical teratoid/rhabdoid tumors (AT/RT) belong to the most common tumors of the central nervous system (CNS) during infancy. They split into four major DNA methylation subtypes: AT/RT-TYR, AT/RT-SHH, AT/RT-MYC, and AT/RT-SMARCA4. Each of these types demonstrates distinct clinical characteristics and gene expression profiles, with AT/RT-TYR most frequently occurring in the fourth ventricle. However, details on the cellular origins and tumor initiation of AT/RT-TYR remain largely unknown and mouse models providing insights into tumor development are completely lacking. Therefore, we aim to identify and characterize the cellular origin of AT/RT-TYR. We performed histopathological examination and bulk- and single-nucleus RNA sequencing analyses across various brain tumor entities. Genetically engineered mouse models with a conditional, Cre/loxP-induced Smarcb1 loss were generated. Here, we show that AT/RT-TYR very often appear intermingled with fourth ventricle choroid plexus (CP) tissue and that tumor cells heavily express CP markers. Analyses of bulk- and single-cell RNA sequencing data reveal a clear resemblance of the AT/RT-TYR to the CP of the fourth ventricle. Finally, Foxj1-cre::Smarcb1fl/fl mice showing loss of Smarcb1 in early CP progenitors gave rise to large, atypical, monociliary CP cells with gene expression most similar to human AT/RT-TYR. In conclusion, analyses of human AT/RT-TYR as well as murine CP cells lacking Smarcb1 point towards the CP of the fourth ventricle as a potential cellular origin of AT/RT-TYR.

P29

Free Neuropathol 6:17:44

Meeting Abstract

Pediatric and AYA meningiomas exhibit distinct molecular and clinical features not reflected in adult-based classification systems

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Background: Meningiomas are the most common primary intracranial tumors in adults but are rare in children and adolescents/young adults (AYA), comprising only 0.4–2.5 % of all cases. Pediatric and AYA meningiomas differ significantly from adult counterparts in epidemiology, anatomical location, histopathology, molecular alterations, and clinical behavior. This study aimed to define the molecular and clinical landscape of meningiomas in patients aged 0–39 years and to evaluate the applicability of adult-derived prognostic frameworks in this population.

Methods: We analyzed 294 meningiomas from patients aged 0–39 years, with 115 patients aged 0–14 and 179 aged 15–39. Integrated analyses included histopathological evaluation, DNA methylation profiling, copy-number analysis, targeted next-generation sequencing (NGS), and clinical outcome assessment.

Results: The cohort showed a high prevalence of *NF2*-driven and *SMARCE1*-altered tumors, often in a hereditary context. In contrast, high-risk alterations common in adult meningiomas, such as *TERT* promoter mutations and

homozygous *CDKN2A/B* deletions, were nearly absent. Established grading parameters based on histology, methylation class, and copy-number alterations failed to reliably predict clinical outcomes. The tumor microenvironment resembled that of adult meningiomas but did not correlate with prognosis. Instead, favorable outcomes were primarily linked to extent of resection and specific chromosomal gains.

Conclusions: Pediatric and AYA meningiomas constitute a biologically distinct subgroup with unique molecular features and clinical behavior. Current adult-based classification and risk stratification systems are inadequate for this population, underscoring the need for age-specific diagnostic and prognostic models.

P30

Free Neuropathol 6:17:46

Meeting Abstract

Case of a myxopapillary ependymoma with multiple relapses and pulmonary metastasis

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Background: Myxopapillary ependymoma (MPE) is a clinically heterogenous disease. While histopathological diagnosis is straightforward, combining histomorphology with HOXB13 immunohistochemistry, predicting the clinical course of MPE is challenging. Approximately half of the patients experience local or even distant recurrences.

Objectives: Investigate prognostic and therapeutic molecular characteristics in MPE in an exemplary case and a small MPE cohort.

Results: We report the case of a 30 year old female patient diagnosed with a presacral MPE which was surgically resected. She suffered from three local relapses 3, 15, and 17 years after primary resection and was treated with repeated resections and adjuvant irradiation. Recently, at the age of 53, the patient was diagnosed with MPE metastasis to mediastinal lymph nodes, pleura and lung. Methylation profiling indicated a strong methylation of the MGMT promotor. Treatment with Temozolomide was initiated.

The methylation profile matched the published subgroup MPE-A which is correlated with a high 10-year relapse rate of 85 % (Bockmayr et al., 2022). Additionally, we detected high expression of PDGFRA in the lung metastasis and the relapses. Our preliminary analyses in a cohort of 25 MPE indicate that PDGFRA is overexpressed in MPE with elevated risk for relapse.

Conclusion: In summary, we present an unusual case of MPE with multiple local recurrences and pulmonary metastasis. Its epigenetic profile of MPE-A is in line with the poor prognosis of this subgroup. First data indicate that expression of PDGFRA in MPE might be an additional marker of a more aggressive biology and a new therapeutic target.

P31

Free Neuropathol 6:17:47

Meeting Abstract

Unravelling ependymoma heterogeneity using integrated proteomic analyses

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Background: Ependymomas (EPN) are heterogeneous tumours occurring across all different ages and the three major compartments of the central nervous system (spine (SP), posterior fossa (PF), supratentorial (ST)), being subdivided into 10 molecular types (WHO 2021), which display distinct clinical characteristics.

Objective(s): Despite advancements in diagnostics and tumour characterisation, the prognosis remains variable and largely dependent on the extent of tumour resection. Thus, there is a high need for targeted adjuvant therapy. Through integrated proteomic analyses, the goal was to identify targetable proteins and putative biomarkers for EPN types.

Method(s): Histomorphology, DNA-methylation-, proteome- and phosphoproteome data was assessed from formalin-fixed paraffin-embedded (FFPE) samples of primary human EPN. Molecular diagnosis was verified based on DNA-methylation data using the brain tumour classifier (V12.8, classifier score > 0.8). Proteome and phosphoproteome data were generated using mass spectrometry. Established epigenomic EPN types were reflected in our main cohort (n = 197 EPN with MPE:n = 40, SP-EPN:n = 31, SP-EPN-MYCN:n = 9, SP-SE:n = 11, ST-SE:n = 16, PFA1:n = 14, PFA2:n = 5, PFB:n = 19, PF-SE:n = 24, EPN-YAP:n = 6, EPN-ZFTA:n = 22).

Result(s): SNF clustering integrating methylome and proteome data revealed stable clustering of EPN types across data modalities. EPN types displayed distinct protein patterns allowing for detection of putative markers and targetable proteins. Results were confirmed in a validation cohort (n = 73). In-depth integrative analyses are ongoing and will help to identify EPN type specific dysregulated biological pathways, biomarkers and treatment targets.

Conclusion(s): EPN types displayed distinct protein patterns allowing for detection of putative markers and targetable proteins. Preliminary findings of the phosphoproteome analysis showed high EPN heterogeneity and overlap with known subtypes.

P32

Free Neuropathol 6:17:49

Meeting Abstract

Identification of a novel type of pineal region tumors with distinct global DNA methylation and SH3TC2 fusions

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Pineal tumors are rare neoplasms, accounting for less than 1 % of all central nervous system (CNS) tumors and occurring primarily in adults. The 2021 World Health Organization (WHO) classification recognizes five distinct tumor entities of primary pineal origin: pineocytoma; pineal parenchymal tumor of intermediate differentiation (PPTID, with two molecular subgroups); pineoblastoma (including four molecular subgroups); papillary tumor of the pineal region (PTPR, with three molecular subgroups); and desmoplastic myxoid tumor of the pineal region, SMARCB1-mutant.

Here, we describe a previously unrecognized tumor type of the pineal region (n = 27) characterized by a unique and consistent DNA methylation profile that does not match any known CNS tumor class and is distinct from all currently recognized pineal tumor entities. The cohort displayed a balanced sex distribution, with a mean patient age of 35 years (range: 7–74 years).

Histopathological analysis revealed features reminiscent of other pineal tumors, including papillary architecture and expression of cytokeratin, synaptophysin, and OTX2. A subset of our tumors also focally expressed desmin, whereas all tumors lacked CRX expression. Notably, 10 of the 13 tumors analyzed harbored a novel *RP11-331K21.1:SH3TC2* gene fusion, which has not previously been reported in any CNS tumor entity.

Taken together, our findings define a novel molecular subgroup of pineal region tumors characterized by *SH3TC2* fusions and a unique DNA methylation signature. Recognition of this group is important for accurate diagnosis and may have implications for future tumor classification and treatment strategies.

P33

Free Neuropathol 6:17:51

Meeting Abstract

TTF-1 expression is associated with a hypomethylation signature in schwannomas

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Background: Thyroid transcription factor-1 (TTF-1/ NKx2.1) is a well-known transcription factor, most notably associated with adenocarcinoma of the lung. Recently, TTF-1 expression has been reported in a subgroup of schwannomas, though its biological and clinical significance remains unclear.

Objectives: This study aims to characterize the epigenetic and clinical features of TTF-1-positive schwannomas.

Questions: Do TTF-1-positive schwannomas exhibit a distinct epigenetic signature? How is this signature characterized? Could TTF-1 expression define a biologically distinct schwannoma subgroup? Is TTF-1 expression associated with clinical features such as localization or growth behavior?

Methods: We performed immunohistochemical, and clinical analyses on 67 schwannomas, including vestibular, spinal, and peripheral schwannomas. TTF-1 expression was assessed by immunohistochemistry. Epigenetic profiling of 24 schwannomas was conducted using the Illumina EPIC 850k array. Bioinformatic analysis was performed using established R packages (ChAMP, limma).

Results: Unsupervised dimensionality reduction using t-SNE and UMAP revealed distinct clustering of TTF-1-positive schwannomas, based on the 1,000 most variable CpG sites. Differential methylation analysis revealed 2,234 CpG sites, predominantly hypomethylated (n = 1,924). Enrichment analysis (KEGG, GO) indicated enrichment of cancer-related pathways (PI3K/AKT/mTOR, MAPK, Hippo) in differentially methylated CpG sites of TTF-1-positive schwannomas. The TTF-1/NKx2.1 locus itself showed no differential methylation but exhibited increased copy number variation, particularly in spinal schwannomas. MIB1 staining suggested higher proliferative activity in TTF-1-positive spinal schwannomas. Limited follow-up data indicated a tendency toward increased facial nerve involvement and proximity to the brainstem.

Conclusions: TTF-1 expression defines a schwannoma subgroup with distinct epigenetic alterations. Further studies are needed to clarify its clinical implications.

P34

Free Neuropathol 6:17:52

Meeting Abstract

Expansion of the spectrum of tumors diagnosed as myxopapillary ependymomas

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Background: For a significant portion of spinal ependymomas (SPE), pathologists reported conflicting results between morphologic diagnosis and the DNA methylation-based classification. A study by the German Glioma Network reported that nearly one third of the histologically diagnosed spinal ependymomas were assigned by methylation to the myxopapillary ependymoma (MPE) class.

Objective(s): We address this topic and focus on SPE cases exhibiting a methylation profile of MPE.

Method(s): We performed immunohistochemical, AI-assisted morphological and methylation analyses on 100 MPEs and SPEs. Mass spectrometry-based proteomic analysis was conducted on 54 of these tumors.

Result(s): Pearson correlation matrix showed higher similarity between discrepant cases and MPEs. Principal component analysis revealed predominant clustering of the discrepant cases with MPEs. Proteomic analysis identified HOXB13 as the most differentially expressed protein between MPE and SPE. Immunohistochemical staining of 100 tumor samples demonstrated 100 % sensitivity and 100 % specificity. HOXB13 showed positive staining in all discrepant cases. The extracellular proteoglycan versican was overrepresented in the myxoid matrix of MPE. Employing digital pathology tools and VCAN immunohistochemistry, we demonstrated that discrepant cases frequently contain myxoid foci. 67 cases that were not in the training cohort of Hetairos, an AI-assisted morphology tool, were evaluated. 16 of the 17 discrepant cases (94 %) had a prediction for MPE.

Conclusion(s): The different layers of information demonstrated that discrepant cases show a pattern more similar to MPE. We therefore propose classifying tumors that resemble morphologically SPE but exhibit a mcMPE profile or with nuclear expression of HOXB13 as MPE irrespective of their morphological appearance.

P35

Free Neuropathol 6:17:53

Meeting Abstract

Addressing MGMT activity in glial cell lines

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The silencing of the O6-Methylguanine-DNA methyltransferase (MGMT) by methylation of its promoter is the most important predictive marker for the current standard chemotherapy in malignant glioma.

However, methylation detection is indirect and other parameters may contribute. Therefore, it is our objective to establish a method for the direct detection of MGMT activity in different samples using synthetic methylated oligonucleotides.

For our method validation we chose commercial glioblastoma cell lines (T98G, U-87 MG) and the MGMT knock-out cell line HAP1 to test their suitability as general models for glioma before transitioning to patient samples.

We characterized them using our molecular diagnostics standard panel for gliomas, which, among others, includes MGMT analysis by pyrosequencing and methylation-specific-PCR. Additionally, we performed qPCR and Western blot. Simultaneously, we quantified the demethylation activity of recombinant MGMT and cell extracts using reverse-phase liquid chromatography (LC).

Our analysis revealed that both cell lines show genetic markers which classify according to WHO as a grade 4 glioblastoma. By pyrosequencing, MGMT-promotor-methylation in HAP1 was ~10 %, T98G 31 % and U-87 MG 54 %. qPCR confirmed successful knock-out and no detectable MGMT-mRNA in U-87 MG, but in T98G. Western blot confirmed these results. The LC-method was developed and optimized with (un)methylated standards and validated using recombinant MGMT: Methylated oligonucleotides (substrate) can be well separated from its demethylated counterparts (product) in submicromolar ranges. We currently test cellular extracts with differing MGMT amounts to mimic complex samples.

A successful transfer to patient samples can support therapy decision using MGMT as a biomarker.

P36

Free Neuropathol 6:17:54

Meeting Abstract

Loss of global DNA methylation is prognostic in oligodendrogliomas

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Background: IDH-mutant gliomas are categorized into IDH-mutant astrocytomas (CNS WHO grades 2, 3, 4) and IDH-mutant, 1p/19q-codeleted oligodendrogliomas (CNS WHO grades 2, 3). Apart from specific subgroups, IDH-mutant and 1p/19q-codeleted oligodendrogliomas are assigned to a single DNA methylation class using the Heidelberg brain tumor classifier, whereas IDH-mutant astrocytomas are divided into two methylation classes: astrocytoma, IDH-mutant lower grade and astrocytoma, IDH-mutant high grade. Occasionally, IDH-mutant and 1p/19q-codeleted oligodendrogliomas are classified as astrocytomas by methylation profiling despite the clear presence of 1p/19q codeletion. This study aims to investigate the significance of this alternative classification.

Method(s): We collected 69 IDH-mutant and 1p/19q-codeleted oligodendrogliomas classified by the Heidelberg brain tumor classifier (v12.8) as either astrocytoma, IDH-mutant, lower grade or high grade. Methylation data were analysed for methylation class assignment and deconvoluted using MethylCIBERSORT to determine cell type proportions. Clinical follow-up data were retrospectively obtained for 40 of the 69 patients.

Result(s): IDH-mutant and 1p/19q-codeleted oligodendrogliomas assigned to the astrocytoma, IDH-mutant high grade methylation class exhibited a loss of global DNA hypermethylation and significantly worse overall survival compared to CNS WHO grade 3 IDH-mutant and 1p/19q-codeleted oligodendrogliomas assigned to the oligodendroglioma methylation class ($p = 0.0019$). Deconvolution revealed a higher proportion of residual brain tissue in oligodendrogliomas classified as IDH-mutant astrocytoma, lower grade compared to other methylation classes of IDH-mutant gliomas.

Conclusion(s): Our findings suggest that decreased global DNA methylation in IDH-mutant and 1p/19q-codeleted oligodendrogliomas, as indicated by methylation classification into the astrocytoma, IDH-mutant, high grade methylation class, is associated with poorer overall survival.

P37

Free Neuropathol 6:17:57

Meeting Abstract

Leveraging peptide-level proteomics to detect brain cancer specific proteoforms

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Background: Conventional bottom-up proteomics approaches rely on protein-group inference. This strategy overlooks peptide-level information of the proteome — particularly the existence of proteoforms, which are distinct molecular species originating from a single gene locus via mechanisms such as alternative splicing, proteolytic cleavage, and post-translational modifications. The commonly overlooked proteoform level is expected to provide clinically valuable information, especially in the context of complex and molecularly heterogeneous diseases such as brain cancers.

Objective(s): This study leverages peptide-level data to identify and characterize differentially regulated proteoform-groups across brain cancer subtypes.

Question(s): Do specific brain cancer subtypes exhibit distinct patterns of proteoform-group regulation? If so, which subtypes are affected, how do these patterns differ from those in other tumor classes, and what molecular mechanisms underlie these regulatory differences?

Method(s): To address these questions, we applied the COPF algorithm (Bludau et al., 2021), which infers proteoform-groups based on peptide correlation patterns, to a clinical mass-spectrometry-based proteomics dataset comprising 31 patient samples across 4 brain tumor subtypes, including astrocytoma, glioblastoma, meningioma and oligodendroglioma.

Result(s): Our analysis revealed multiple proteins with differentially regulated proteoform groups across brain cancer types. Notably, RTN4 (Nogo) and LIMA1 showed differential proteoform patterns in meningioma compared to other subtypes, matching known RNA splice isoforms, that would have been missed in conventional proteomics data analysis.

Conclusion(s): Proteoform-group inference can reveal subtype-specific expression patterns, highlighting its utility for uncovering molecular diversity in brain cancer and the identification of novel biomarkers.

P38

Free Neuropathol 6:17:58

Meeting Abstract

Glioblastoma-derived secreted proteins drive invasion and affect neuronal integrity

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Background: Glioblastoma (GB) is an aggressive brain tumor characterized by diffuse infiltration and reciprocal interactions with the surrounding brain tissue, including neurons. Understanding how tumor-derived factors contribute to both invasion and neuronal disruption is critical to elucidating glioma-brain interactions.

Objective: To identify glioma-derived secreted proteins that promote tumor cell invasion and to investigate whether these factors also affect the structural and functional integrity of human neurons.

Methods: We combined two complementary screening approaches in patient-derived glioma stem-like cells (GSCs): (1) serial selection of highly invasive subpopulations, and (2) a genome-wide CRISPR activation (CRISPRa) screen. Candidate factors were validated via overexpression and CRISPR/Cas9-mediated knockout across multiple GSC lines. Functional consequences were assessed using 2D assays and 3D cerebral organoids derived from human induced pluripotent stem cells (hiPSCs), including live-cell imaging in the Glioma Cerebral Organoid (GLICO) model. Tumor-neuron interactions were studied using a competition assay with fluorescently labelled wildtype and knockout cells in co-culture with hiPSC-derived neurons, and via direct exposure of mature neurons to recombinant proteins.

Results: CHI3L1 and IGFBP5 were identified as secreted regulators of glioma invasion, enriched at the tumor invasive front. Functional assays demonstrated consistent effects on cell motility and infiltration. Exposure of neurons to recombinant CHI3L1 or IGFBP5 led to synaptic alterations and neurotoxic effects.

Conclusion: CHI3L1 and IGFBP5 are secreted drivers of glioma invasion and modulators of neuronal integrity. These findings underscore the importance of tumor-neuron interactions, particularly at the invasive front where single tumor cells interface with intact brain parenchyma.

P39

Free Neuropathol 6:17:59

Meeting Abstract

Establishment of Droplet Digital™ PCR (ddPCR™)-based assays for the diagnostic detection of MGMT promoter methylation in malignant gliomas

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MGMT promoter methylation is the most relevant predictor of response to chemotherapy with temozolomide (TMZ) in patients with newly diagnosed IDH-wildtype glioblastoma. In routine diagnostics, the *MGMT* promoter methylation status is commonly assessed by either pyrosequencing (PSQ) of bisulfite-modified DNA or global EPICv2 bead array-based DNA methylation profiling using the STP27 algorithm. We aimed to establish and validate ddPCR™ as a novel method to allow for rapid diagnostic detection of the methylation status at relevant CpG sites in the *MGMT* promoter-associated CpG98 island. Results obtained by ddPCR™ were compared to those obtained by PSQ or STP27. In total, we established three different duplex-ddPCR™ assays to analyse the methylation status at selected CpG sites covering (1) four of the CpG sites commonly analysed by PSQ (CpGs 76–79, numbering of CpG sites in the *MGMT*-associated CpG98 island according to Malley *et al*., Acta Neuropathol. 2011, 121:651–661), as well as (2) CpG30 and CpG31, and (3) CpG84, with CpG31 corresponding to CpG10, and CpG84 to CpG16 of the MGMT-STP27 algorithm. Comparison of the newly established ddPCR™-based assays with results obtained by PSQ or EPICv2-based STP27 analysis showed a high degree of consistency in the methylation levels detected at the respective CpG sites. Moreover, differences in methylation status at the distinct CpG sites interrogated by either PSQ or STP27 were reproduced by the distinct ddPCR™ assays. Our results thus implicate ddPCR™ as a novel, rapid, comprehensive and quantitative approach for the routine diagnostic determination of the *MGMT* promoter methylation status in malignant gliomas.

P40

Free Neuropathol 6:17:60

Meeting Abstract

Exploring glioblastoma through CpG site-specific methylation and transcription factor occupancy

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Glioblastoma (GB) is a highly malignant primary brain tumor that shows extensive heterogeneity and invasiveness. While several studies highlight the role of epigenetic alterations in GB aggressiveness and heterogeneity, the functional consequences of individual CpG site methylation remains poorly understood. Methylation at CpG sites can influence transcription factors (TF) binding, thereby affecting gene regulation and potentially impacting tumor cell behavior and interactions within the tumor microenvironment (TME), including invasion.

The present study investigates the impact of differential CpG site-specific methylation on TF occupancy and its role in regulating the expression of Chitinase 3-like-1 (CHI3L1), a gene differentially expressed in highly invasive GB stem cells (GSCs), aiming to examine the role of methylation in TF binding sites and its influence on GB invasiveness.

This project employed methylation analysis utilizing Illumina EPIC 850k array across various GB cell lines, revealing hypomethylation of CHI3L1 promoter in more invasive GSCs. To assess the functional relevance of this finding to promoter activity, site-directed mutagenesis of the specific CpG sites was performed, followed by luciferase reporter assay. *In silico* motif enrichment analyses identified TF binding motifs around the candidate CpG sites, suggesting that methylation status could modulate TF binding. Finally, chromatin immunoprecipitation (ChIP)-qPCR experiments were established to confirm TF binding at differentially methylated CpG sites.

Future work will examine the role of these TFs in differential expression of other genes involved in GB invasiveness using ChIP sequencing. Additionally, we seek to optimize the ChIP method for formalin-fixed paraffin-embedded tissue, as they are more physiologically relevant models.

P41

Free Neuropathol 6:17:61

Meeting Abstract

Integrated analyses reveal four distinct molecular subgroups in corticotroph pituitary neuroendocrine tumors/adenomas

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Background: Corticotroph pituitary neuroendocrine tumors (PitNETs/adenomas) are common sellar neoplasms with variable clinical presentations. According to the current WHO classification, tumor subtyping is based on histopathology and comprises subtypes with either sparse or dense granulation as well as Crooke cell tumors. Previous studies on confined case series have reported three distinct molecular profiles in corticotroph PitNETs. These molecular profiles primarily related to *USP8*-mutation status and GATA3-expression levels, while their alignment with the established WHO subtypes was limited.

Objective: In this ongoing work, we aim to further explore molecular subgroups of corticotroph PitNETs and their clinical relevance.

Methods: We compiled previously published and publicly available global epigenomic and/or transcriptomic data of 171 corticotroph PitNETs, derived from a total of 7 independent studies. Gap statistics and consensus clustering were applied to delineate molecular subgroups within the compiled datasets.

Results: Based on epigenomic as well as transcriptomic data, corticotroph PitNETs separated into four distinct molecular subgroups, preliminarily named subgroups 1–4. *USP8*-wildtype and GATA3-negative tumors split into subgroups 1 and 2. *USP8*-wildtype tumors with increased GATA3 expression were mainly found in subgroup 3. *USP8*-mutated tumors with increased SSTR5 expression were predominantly aggregated in subgroup 4. Preliminary investigations indicated that these four molecular subgroups enable improved prognostic stratification beyond current concepts.

Conclusions: Our findings demonstrate that corticotroph PitNETs segregate into four molecular subgroups. Ongoing investigations including detailed clinicopathological characterizations of each subgroup have the potential to improve the existing histopathological classification framework in corticotroph PitNETs.

P42

Free Neuropathol 6:17:63

Meeting Abstract

Harnessing stress adaptive response mechanisms for pharmacological targeting of cancer

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Brain cancer cells are subjected to metabolic stress, such as hypoxia and glucose restriction, due to defective tumor vasculature. This forces brain cancer cells to evolve adaptive mechanisms to reprogram their metabolism, which includes inhibition of mRNA translation — a highly energetic process. The rate of mRNA translation is controlled by the mechanistic target of rapamycin (mTOR)/ eukaryotic initiation factor 4E binding protein (4EBP) pathway according to glucose concentrations. Glucose starvation blocks mTOR, which in turn leads to 4EBP1 activation. This results in the inhibition of mRNA translation initiation as 4EBP1 binds to and blocks the translation initiation factor eIF4E. We found that high 4EBP1 expression is a factor of poor prognosis in glioblastoma and medulloblastoma. Furthermore, we demonstrated that 4EBP1 protects glioblastoma and medulloblastoma cells under glucose starvation and promotes tumorigenicity both in vitro and in vivo. These findings support that 4EBP1 may be a potential therapeutic target in glioblastoma and medulloblastoma. Therefore, we are aiming to develop a targeting strategy against 4EBP1 to drive cancer cells to death under metabolic stress. Using an in-silico screening approach we identified potential inhibitors of 4EBP1, predicted by molecular docking to disrupt the 4EBP1-eIF4E interaction. Using in vitro binding assays and cellular assays, these compound candidates are being validated for their ability to block the physical binding of 4EBP1 to eIF4E, to induce cell death under glucose deprivation and to reduce tumorigenic potential. Our strategy to target 4EBP1 may represent a novel therapeutic approach to treat, yet, incurable malignant brain tumors.

P43

Free Neuropathol 6:17:64

Meeting Abstract

Mechanisms of transformation from subependymoma to ependymoma

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Background: Subependymomas (SEs) are slow-growing central nervous system tumors, yet those in the posterior fossa (PF-SEs) can behave more aggressively, often showing areas of higher-grade ependymal differentiation. Recent studies suggest that chromosome 6 loss and TERT promoter mutations are associated with this transition, supporting a model of progression from low-grade to more malignant phenotypes. This study investigates the molecular and spatial heterogeneity underlying this evolution.

Methods: We analyzed 27 PF-SE samples from 25 patients, alongside 5 PF-A and 6 PF-B ependymomas. Using single-nucleus RNA sequencing, spatial transcriptomics, and bulk RNA sequencing, we assessed transcriptional and spatial dynamics. Hypoxia-inducible factor 1-alpha (HIF1A) immunohistochemistry was also performed.

Results: A hypoxia-associated transcriptional program was enriched in ependymal-like regions of PF-SEs. Copy number analysis revealed increasing chromosome 6 loss correlating with ependymal differentiation and hypoxia-related gene expression. Tumors with both subependymal and ependymal areas exhibited a reactive transcriptional state marked by stress and interferon signaling, suggesting an active transitional phase. Immune profiling showed a shift from pro-inflammatory to immunosuppressive signatures along the histologic spectrum, indicating progressive immune evasion.

Conclusion: These findings highlight hypoxia and chromosome 6 loss as interconnected mechanisms driving malignant transformation in PF-SEs. The parallels with aggressive PF-As suggest shared evolutionary pathways among posterior fossa ependymal tumors.

P44

Free Neuropathol 6:17:65

Meeting Abstract

Leveraging off-target reads for genome-wide copy number profiling in NGS panels

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Background: Copy number variations (CNVs) are crucial for the diagnosis and prognosis of central nervous system (CNS) tumors. Although typically assessed using chromosomal or DNA methylation microarrays, CNV data can also be extracted from next-generation sequencing (NGS) panels. Off-target reads mapping outside the targeted regions are usually discarded in standard NGS workflows.

Objectives: Our study evaluates whether these reads can be repurposed for genome-wide CNV profiling in CNS tumors using a small, custom NGS panel not specifically designed for CNV analysis.

Methods: We analyzed 60 CNS tumors, including IDH-wildtype glioblastomas (n = 25), oligodendrogliomas (n = 15), ependymomas (n = 9), medulloblastomas (n = 6), and choroid plexus tumors (n = 5). Each case underwent EPIC methylation profiling and hybrid-capture NGS panel (31 genes, 0.17 Mb). CNV profiles were generated using CNVkit and custom Python scripts.

Results: Sequencing yielded an average of 26.5 million reads per sample (range: 5.1–72.2 million), with a mean off-target rate of 64.9 % (range: 45–92 %). EPIC data identified 260 chromosomal arm gains and 271 losses. NGS-derived CNVs showed strong concordance (average R = 0.915, p = 0.000014). Hallmark alterations, including +7/−10 and 1p/19q codeletion, were reliably detected from off-target reads. Nineteen focal amplifications were concordantly identified, including MDM4 and MYCN, which were not covered by the panel. Of 20 homozygous deletions identified by the array, 19 (95 %) were also observed in the NGS data.

Conclusion: Taken together, our findings demonstrate the feasibility of using off-target reads for CNV profiling in CNS tumors, enabling detection of clinically relevant events even outside targeted regions.

P45

Free Neuropathol 6:17:66

Meeting Abstract

Establishing ultra low-input spatial proteomics for glioblastoma analysis

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Background: Glioblastoma shows pronounced spatial heterogeneity that impacts its aggressive behavior and therapy resistance. High-resolution spatial proteomics is needed to characterize distinct tumor microenvironments at the protein level, which cannot be fully captured by transcriptomics alone.

Objective(s): To establish an ultra-low-input spatial proteomics workflow enabling the identification of thousands of proteins from minute, laser-microdissected glioblastoma tissue regions. This approach aims to facilitate detailed molecular profiling of heterogeneous tumor areas.

Question(s): Can this workflow detect spatial protein gradients within glioblastoma microenvironments? How can such data support future investigations of biologically relevant tumor niches?

Method(s): Glioblastoma sections are annotated after H&E staining, followed by precise laser microdissection (~30,000 µm² equivalent to ~250 cell bodies) of morphologically distinct tumor regions. Proteins are analyzed via highly sensitive LC-MS/MS with an optimized ultra-low-input protocol.

Result(s): Using this workflow, we are able to identify approximately 3,500 proteins per microdissected region. This high sensitivity and spatial resolution provide a powerful basis for future applications, such as analyzing perinecrotic zones to explore spatially resolved protein expression patterns relevant to tumor progression.

Conclusion(s): The established ultra-low-input spatial proteomics workflow offers a robust platform for investigating glioblastoma heterogeneity at the protein level. It enables detailed studies of tumor microenvironments and supports the discovery of spatially distinct biomarkers and therapeutic targets.

P46

Free Neuropathol 6:17:67

Meeting Abstract

Comparison of diffuse glioma types with gliomatosis cerebri growth pattern in adult patients

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Gliomatosis cerebri (GC) type 1 is characterised by diffuse infiltration of glioma cells spanning three or more cerebral lobes without contrast enhancement. Its prevalence and clinical relevance across glioma subtypes remain incompletely understood. In a cohort of 853 gliomas epigenetically profiled between 2017 to 2025, GC was found in 1 % of glioblastomas, *IDH* wildtype (GBM; 5/587), 4.5 % of oligodendrogliomas, *IDH*-mutant and 1p/19q-codeleted (4/88), 2.8 % of astrocytomas, *IDH*-mutant (3/108), 4 % of diffuse paediatric-type high-grade gliomas, *H3*-wildtype and *IDH*-wildtype (2/54), and 69 % of the novel glioma type "gliomatosis cerebri-like gliomas, *IDH*-wildtype" (GCLG, 11/16). Gliomas exhibiting the GC phenotype showed significantly higher MGMT promoter methylation rates compared with non-GC tumours (e.g., 46 % vs. 28 % in GBM), whereas MGMT promoter methylation was uncommon in GCLGs with GC growth pattern (7 % vs. 28 %, $p < 0.001$). *IDH*-mutant

gliomas with GC were more often treated with radiotherapy or chemotherapy alone. Notably, GBMs and GCLGs with GC exhibited comparable radiochemotherapy rates (88 % vs. 86 %), while monotherapy and watchful waiting were infrequently employed (0 % vs. 7 % and 12 % vs. 7 %). Outcome for GCLG with GC was significantly better compared to GBM with GC (mPFS 25 vs 12 months, mOS 49 vs 19 months, $p < 0.05$). In summary, while GC is uncommon in most glioma types, it represents the predominant growth pattern in GCLG. Despite infrequent MGMT promoter methylation, the prognosis of GCLG with GC is considerably more favourable than GBM. We therefore recommend comprehensive epigenetic profiling in patients presenting radiologically with GC type 1.

P47

Free Neuropathol 6:17:69

Meeting Abstract

Immunohistochemical expression and differential methylation of HOXB13 reliably distinguishes myxopapillary ependymoma from spinal ependymoma

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Background: Histological distinction of spinal ependymoma from myxopapillary ependymoma may be difficult in individual cases, especially in tumors located in the lumbar region. According to the WHO classification of 2021 unresolved lesions require global DNA methylation profiling for correct classification. Recently, high expression of the homeobox gene *HOXB13* at the mRNA and protein levels has been reported in myxopapillary ependymoma.

Objective(s): We evaluated the diagnostic role of HOXB13 immunostaining in an institutional cohort of patients with spinal neoplasms (n = 143), including different types of spinal ependymal tumors from various locations and other relevant differential diagnoses.

Method(s): Expression of HOXB13 protein was compared to molecular findings obtained by DNA methylation profiling, targeted methylation analysis, and next generation sequencing.

Result(s): Collectively, our findings indicate that strong nuclear HOXB13 immunopositivity is a specific diagnostic marker for myxopapillary ependymoma which enables reliable differentiation of spinal ependymoma, especially in lumbar spinal cord tumors whose precise classification otherwise would require DNA methylation profiling. We additionally provide evidence for differential methylation of *HOXB13*-associated CpG sites and established a pyrosequencing-based assay to interrogate a *HOXB13*-associated CpG site that showed consistent differential methylation between spinal ependymoma and myxopapillary ependymoma.

Conclusion(s): Thus, immunohistochemistry for HOXB13 and/or targeted DNA methylation analysis may constitute fast, resource-friendly approaches to substitute for global DNA methylation profiling in the precise classification of spinal ependymal tumors.

P48

Free Neuropathol 6:17:71

Meeting Abstract

Spatial mapping of immunogenic niches in melanoma brain metastases

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Background: Tumor development and progression are dynamic, time-dependent processes influenced by genetic alterations, environmental factors, and spatial mechanisms - all shaped by interactions with the tumor microenvironment (TME). Resistance to immune checkpoint inhibition (ICi) significantly contributes to the emergence and metastatic progression of brain metastases (MBM) in approximately 40–60 % of melanoma patients.

Objective(s): We employed spatial transcriptomics at the single cell level to investigate spatially defined immunosuppressive programmes in MBM and their role in therapy resistance.

Question(s): What are the spatial characteristics and cellular compositions of immune-suppressive niches in MBM, and how do they relate to resistance against immune checkpoint inhibition?

Method(s): We applied Xenium (10X Genomics) spatial transcriptomic technology to 14 MBM specimens representing various stages of tumor progression and treatment history. Single-cell resolution data were analyzed to deconvolute the immunogenic landscape and cellular phenotypes within the tumor microenvironment.

Result(s): We identified substantial cellular heterogeneity across MBM samples and highlighted tumor subsets expressing BZW2, SOX4, or TAP1. $BZW2^+$ and $SOX4^+$ tumor cells were associated with immune-suppressive microenvironments characterized by poor infiltration of immune cells and tumor-associated macrophages/microglia (TAMs). BZW2 and TAP1 expression showed an inverse correlation, with TAP1 being enriched in "hot" immune cell–infiltrated niches, consistent across tumor stages.

Conclusion(s): Spatially-defined immunosuppressive programs critically shape immune cell infiltration patterns in MBM and may influence the response to immune checkpoint therapies. Understanding these spatial dynamics could inform targeted therapeutic strategies to overcome ICi resistance.

P49

Free Neuropathol 6:17:72

Meeting Abstract

Targeting phosphorylation events in glioblastoma: a semi-spatial (phospho)proteomic workflow for diffuse glioma FFPE samples

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Glioblastoma is the most frequent and aggressive among the glioma. Despite DNA methylation has improved tumor classification, and dissected different subtypes, glioblastoma is still characterized by traditional therapeutic options, resulting in poor patient response and median 18 months survival. EGFR kinase amplification is observed in 60 % of glioblastoma, causing hyperactivation of key signaling pathways regulating proliferation, motility, and apoptosis, thereby contributing to cancer progression. In a fraction of patient, EGFR activation is observed in the absence of EGFR amplification, thus the dissection of EGFR phosphorylation cascade is critical to characterize GBM biology. Phosphorylation events are dynamic, and due to the very low abundance of phosphopeptides, phosphoproteomic analysis is particularly challenging, especially in formalin-fixed paraffin-embedded (FFPE) clinical tissues. Overcoming these obstacles relies on robust phosphopeptide enrichment methods and highest sensitivity mass spectrometers coupled with in-depth computational data analysis pipelines.

In this context, our team at the Division of Neuropathology in Heidelberg has implemented a low-input phosphoproteomic workflow optimized for single punches of FFPE material. The robust and automatized phosphopeptide enrichment strategy highly improves the quantification of phosphorylated peptides in clinical samples, while the downstream bioinformatic analysis enables the systematic mapping of kinase-substrate relationships and elucidates on signaling networks deregulated in glioblastoma.

Our optimized phosphoproteomic pipeline dramatically increases our understanding of glioblastoma biology while identifying patients with active signaling in the absence of target amplification, and holds promise for a more personalized and effective intervention for glioblastoma patients.

P50

Free Neuropathol 6:17:73

Meeting Abstract

Meningioma sanity check: increasing classifier credibility through prototypical feature statistics

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Background: Meningiomas are the most common primary brain tumors. DNA methylation-based classification has significantly improved diagnostic accuracy and prognostication. However, current classifiers — such as the DKFZ Heidelberg Classifier — operate largely as black boxes, providing limited insight into how representative or atypical an individual case is within its predicted class. Enhancing classifier transparency is essential for clinical trust and interpretability.

Methods: We analyzed 2,300 meningioma samples profiled with the Illumina EPIC array platform, including all cases classified into a meningioma subtype, regardless of confidence score. Fingerprinting techniques were applied to exclude recurrent samples. For each subtype, we computed features statistics across multiple axes: CNV profiling (conumee2), tumor microenvironment (MethylCIBERSORT, MethyResolver, EpiDISH, MDBrainT), age, differential methylation regions (DMRs) and OncoTree-based CNV mapping. Dimensionality reduction (t-SNE) and Gradient Boosting Machine (GBM) were used to assess subtype separability. A composite *prototypicality score* is being developed to quantify how typical a case is within its predicted class.

Results: Subtype assignments were consistent across methods and reflected distinct biological profiles. CNV analyses uncovered subtype-specific alterations correlating with clinical features. Deconvolution identified heterogeneous immune and stromal cell populations. Preliminary GBM models demonstrated predictive performance for subtype assignment.

Conclusion: This large-scale methylation study enhances understanding of methylation-based meningioma classification and supports the development of trust-enhancing tools. Ongoing work aims to implement a prototypicality score and provide a web interface for classifier inspection and visualization from uploaded .idat files and clinical metadata.