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Neuropathology

Prepared by the Association for Molecular Pathology Training and Education Committee

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Diffuse glioma

Adult-type diffuse gliomas

Astrocytoma, IDH-mutant

- *IDH1* mutation in codon R132; *IDH2* mutation in codon R172
- *TP53* alteration (consider germline testing for Li-Fraumeni in appropriate clinical context)
- Frequent *ATRX* inactivating alterations (typically mutually exclusive with 1p/19q co-deletion and *TERT* promoter mutation)
- Homozygous *CDKN2A/B* deletion associated with poorer prognosis and diagnostic of grade 4; inactivating mutations may also portend worse prognosis

Oligodendroglioma, IDH-mutant and 1p/19q-codeleted

- Diagnosis requires *IDH1* mutation in codon R132 or *IDH2* mutation in codon R172 and whole arm loss of 1p and 19q [FISH, CGH, SNP-array, NGS]
- *TERT* promoter mutation in nearly all cases (mutually exclusive with *ATRX*)
- *CIC* mutation (up to 70%)
- *FUBP1* mutation (20-30%)

Glioblastoma, IDH-wildtype

- Must be *IDH1/2* wildtype and H3-wildtype (i.e. not H3K27-altered or H3G34-mutant)
- Molecular features diagnostic of glioblastoma, irrespective of histology:
  - *TERT* promoter mutation (NB: may occur in other tumor types, e.g. PXA)
  - *EGFR* amplification (36%)
  - Gain of whole chromosome 7 and loss of whole chromosome 10 (59%)
- Frequent alterations:
  - *CDKN2A/B* loss, 13q/22q loss, 19/20 gain
  - *EGFR* mutations, gains, fusions (60%)
    - *EGFRvIII* (intragenic deletion exons 2-7), *EGFR* fusion with diverse partners
  - *PDGFRA* alts (10-15%), *MET* alts (2-5%), *FGFR3* (3%)
    - *FGFR3::TACC3* fusion have better prognosis, oligodendroglial histology
  - *MDM2/MDM4* gain (15%)
  - *TP53* alteration (20-25%)
  - *CDK4/CDK6* amp (15%)
  - *RB1* alteration (8%)
- *NTRK1/2/3* fusions (1-2%)
- *BRAF* V600E- occurs in epithelioid GBM (overlap with PXA)
- Elevated TMB- may result from temozolamide hypermutation. Consider Lynch and constitutional mismatch repair deficiency
- *MGMT* promoter methylation (40-50%) predicts better outcomes with temozolamide

Pediatric-type diffuse gliomas

Pediatric-type low-grade gliomas

- *MYB::QKI* fusion associated with angiocentric glioma
- *MYB* or *MYBL1* fusion, often with *PCDHGA1*, *MMP16*, and *MAML2*, associated with Diffuse astrocytoma, *MYB*- or *MYBL1*- altered (must be IDH/H3 wildtype)
- Polymorphous low grade neuroepithelial tumor of the young (PLNTY) associated with MAPK-alterations, often *FGFR2/3* fusions or *BRAF* V600E
- Diffuse low grade glioma, MAPK-altered associated with ITD/TKD of *FGFR1* or *BRAF* V600E; lack *CDKN2A/B* alterations and *IDH*/H3 wildtype

Pediatric-type high-grade gliomas

Diffuse midline glioma, H3K27-altered

- Diagnosis requires *IDH1/2* wildtype
- Various histone isoforms may harbor H3K28M alteration (H3.3, H3.1, H3.2), thus various gene targets most common gene altered is *H3F3A*
  - Note that alterations may also occur in other tumor types, e.g. pilocytic astrocytoma, ganglioglioma and therefore must be diffusely infiltrating and midline for DMG diagnosis
- Often co-occurring mutations that are specific to location
- *TP53* mutation associated with radioresistance
- *EGFR* exon 20 indels also associated with DMG
- A new subtype, H3K27 & *BRAF/FGFR1*-coaltered DMG, has a better outcome

Diffuse hemispheric glioma, H3G34-mutant

- Missense mutation in *H3F3A* (H3-3A)- p.G35R, p.G35V

Diffuse pediatric high-grade glioma, H3-wildtype and IDH-wildtype

- Definitive diagnosis by methylation profiling and appropriate clinicopathologic context
- Three molecular subgroups:
  - pHGG RTK1: enriched for *PDGFRA* amplification (33%)
  - pHGG RTK2: enriched for *EGFR* amplification and *TERT* promoter mutations (50-64%)
  - pHGG MYCN: enriched for *MYCN* amplification (50%)

Infant-type hemispheric glioma

- Typically associated with RTK-fusions- *ALK*, *ROS1*, *MET*, *NTRK1/2/3*

Circumscribed glioma

- Pilocytic astrocytoma associated with MAPK pathway alterations:
  - *KIAA1549::BRAF* fusion (>60%)
  - *BRAF* mutations (5-10%)
  - Other *BRAF* fusions (<5%)
  - *NF1* mutation (5-10%)
  - *FGFR1* mutation (<5%)- frequently midline location
  - *NTRK1/2/3* fusions (~2%)
- High-grade astrocytoma with piloid features:
  - Formerly anaplastic pilocytic astrocytoma
  - MAPK pathway alteration in association with:
    - Homozygous *CDKN2A/B* deletion
    - *ATRX* inactivating alteration
- Pleomorphic xanthoastrocytoma (PXA):
  - IDH- and H3-wildtype
  - Overlap with epithelioid glioblastoma
  - *BRAF* V600E most common (if not, other MAPK pathway alterations)
  - Homozygous *CDKN2A/B* deletion
  - *TERT* promoter mutation in a subset
- Subependymal giant cell astrocytoma (SEGA): Biallelic inactivation of *TSC1* or *TSC2*
- Chordoid glioma: *PRKCA* p.D463H
- Astroblastoma, *MN1*-altered: *MN1* fusion with *BEND2* or *CXXC5*

Ependymal tumors

- Supratentorial ependymomas:
  - *ZFTA*-fused ependymoma (formerly *RELA*-fused ependymoma)
    - *ZFTA* (formerly *C11orf95*) frequently fused to *RELA*
    - *CDKN2A/B* deletion associated with poor prognosis
  - *YAP1*-fused ependymoma (often *YAP1::MAMLD1*)
- Posterior fossa ependymomas:
  - Divided into PFA-A and PFA-B by methylation profiling
  - 1q gain- worse prognosis
- Spinal ependymoma:
  - Frequent loss of 22q, *NF2* mutation, absent *MYCN* amplification
- Spinal ependymoma, *MYCN* amplified: *MYCN* amplification diagnostic and associated with worse prognosis
- Myxopapillary ependymoma:
  - Recurrent gains of chromosome 16 and losses of chromosome 10
- Subependymoma:
  - Recurrent losses involving chromosome 19, partial loss of chromosome 6, *TRPS1* mutation
  - Rarely *H3K28M* mutation

Glioneuronal/ neuronal tumors

- Ganglioglioma:
  - MAPK-pathway alterations including *BRAF* V600E (10-60%), *BRAF* R506 mutations (10%), *BRAF* fusions (e.g. *KIAA1549::BRAF* in spinal cord), *RAF1* fusion, *KRAS* mutation, *NF1* inactivation
- Desmoplastic infantile astrocytoma (DIA)/Desmoplastic infantile ganglioglioma (DIG):
  - MAPK-pathway alterations- often *BRAF* or *RAF1* mutation/fusions
- Dysembryoplastic neuroepithelial tumor (DNET)
  - Activating alterations in *FGFR1*- ITD, fusion, mutation (40-80%)
  - Also seen in setting of RASopathies, e.g., Noonan, neurofibromatosis
- Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC): monosomy 14 in vast majority
- Papillary glioneuronal tumor: *PRKCA* gene fusion (typically *SLC44A1::PRKCA*), 17q focal gain in subset
- Myxoid glioneuronal tumor: *PDGFRA* p.K385 dinucleotide mutation
- Diffuse leptomeningeal glioneuronal tumor (DLGNT): MAPK alteration (commonly *KIAA1549::BRAF* fusion), 1p deletion
  - 1q gain may portend worse prognosis
- Multinodular vacuolating neuronal tumor (MVNT): MAPK-pathway activating mutations- *MAP2K1*, *BRAF* (excluding *BRAF* V600E), *FGFR2* fusions
- Extraventricular neurocytoma: *FGFR1::TACC1* fusions (60%)
- Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease): Associated with Cowden syndrome (usually *PTEN* germline alteration)
- Cerebellar liponeurocytoma: Frequent losses involving 2p and 14

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Embryonal tumors

Medulloblastoma

- Four main molecular subgroups, but can be further sub grouped
- WNT-activated (10%)
  - *CTNNB1*, *DDX3X* mutation; monosomy 6
  - Consider germline *APC* mutation
- SHH-activated, *TP53* wildtype:
  - *PTCH1* mutation/deletion, 10q loss, *SMO*, *SUFU*, *ELP1*, *DDX3X*, *KMT2D* mutations
  - Consider germline *PTCH1*, *SUFU*, *ELP1* alterations
- SHH-activated, *TP53*-mutant
  - *MYCN*/*GLI2* amplification, 17p loss
  - *TP53*, *DDX3X*, *TERT* mutation
  - Consider germline *TP53* alteration
- Non-WNT/non-SHH, group 3
  - *MYC*/ *MYCN* amplification, 1q/7 gain, 10q, 16q loss, isodicentric 17q
  - Consider *BRCA2*, *PALB2* germline alterations (rare)
- Non-WNT/non-SHH, group 4
  - *MYCN*/*OTX2*/*CDK6* amplification
  - 7 gain, 8/11 loss; isodicentric 17q
  - *KDM6A*, *ZMYM3*, *KMT2C*, *KMT2D*, *KBTBD4* mutation
  - Consider *BRCA2*, *PALB2* germline alterations (rare)

Atypical teratoid/rhabdoid tumor

- Biallelic inactivation of *SMARCB1* or *SMARCA4* (<5%)
- 3 subtypes by methylation profiling: AT/RT-SHH (44%), AT/RT-TYR (34%), AT/RT-MYC (22%) with different clinical outcomes and localization

Embryonal tumor with multilayered rosettes (ETMR)

- Structural alteration of a microRNA cluster on 19q13.42 (C19MC) (90%)
  - Broad chromosome 2 gain
- ~half of cases without C19MC have *DICER1* mutation (consider germline testing)

CNS neuroblastoma, FOXR2-activated

- *FOXR2* structural rearrangement/ gene fusion or methylation profile

CNS tumor with BCOR internal tandem duplication (ITD)

- *BCOR* ITD involving exon 15
- Not to be confused with *BCOR/BCORL1*-fused gliomas

Pineoblastoma

- Four main subtypes by methylation profiling
- *RB1* alterations (consider germline *RB1*, trilateral retinoblastoma)
- *DICER1*, *DROSHA*, *DGCR8* alterations (consider *DICER1* syndrome)

Nerve sheath tumors

- Schwannoma: *NF2* (50-75% sporadic), *SOX10* indels (30% sporadic), germline *SMARCB1* or *LZTR1*
- Neurofibroma: biallelic inactivation of *NF1*
  - Atypical Neurofibromatous neoplasm with uncertain biologic potential (ANNUBP) associated with *CDKN2A/B* deletion
  - Malignant peripheral nerve sheath tumor (MPNST): *CDKN2A/B* deletion, *SUZ12*/*EED* mutations, *NF1* loss, and complex genome. High-grade MPNST shows loss of H3K27me3 (50-80%)
- Hybrid nerve sheath tumors: *ERBB2* mutations in subset
- Malignant melanotic nerve sheath tumor- *PRKAR1A*

Melanocytic neoplasms

- Primary melanocytic neoplasms will lack UV mutational signature unlike a subset of high TMB skin melanomas
- Uveal melanomas: *GNAQ*, *GNA11*, *SF3B1*, *BAP1*, *EIF1AX* mutations
- Primary meningeal melanomas: *GNAQ*, *GNA11*, *PLCB4*, *CYSTLR2*
  - *SF3B1*, *EIF1AX*, *BAP1* mutations, monosomy chromosome 3, complex copy number profile, may portend worse prognosis
  - In children, primary meningeal melanomas associated with *NRAS*/*BRAF* mutations

Mesenchymal tumors

- Solitary fibrous tumor: *NAB2*::*STAT6* fusion, *TERT* promoter mutation associated with poorer prognosis
  - Challenging to detect by PCR/FISH; NGS/IHC preferred
- Alveolar rhabdomyosarcoma: *PAX3*::*FOXO1*, *PAX7*::*FOXO1*
  - May be OLIG2 positive
- Intracranial mesenchymal tumor, FET::CREB fusion positive:
  - *EWSR1* or rarely *FUS* (FET) fused with *CREB1*, *ATF1*, *CREM* (CREB family)
- *CIC*-rearranged sarcoma:
  - *CIC* fused with *DUX4*, *FOXO4*, *LEUTX*, *NUTM1* (NUT+ by IHC), *NUTM2A*
- Primary intracranial sarcoma, *DICER1*-mutant:
  - Pathogenic *DICER1* inactivating mutation (somatic or germline)
- Ewing sarcoma:
  - FET gene member (usually *EWSR1*, rarely *FUS*) fused with ETS family gene member (often *FLI1*, *ERG*, *ETV1*, *ETV4*, *FEV*)
  - *EWSR1*::*FLI1* accounts for 85% cases (t11:22)(q24;q12)
  - *STAG2*, *CDKN2A*, *TP53* alterations may portend worse prognosis
- Desmoplastic small round cell tumor:
  - Rare in CNS
  - *EWSR1*::*WT1* fusion
- Mesenchymal chondrosarcoma: *HEY1*::*NCOA2* fusion
- Chondrosarcoma: *IDH1/2* mutation (60%)
- Chordoma: *TBXT* duplication (27%), *PIK3CA* mutation (16%), *LYST* mutation (10%)
  - *SMARCB1* alteration in poorly differentiated chordoma

Meningioma

- Genomics influenced by tumor location, type of meningioma and may predict outcome
  - Most frequent abnormality is monosomy 22q / *NF2* mutation
- Spinal: *NF2*/22q loss
- Skull base: *TRAF7*, *AKT*, *SMO*, *PIK3CA*
- Secretory meningiomas: *TRAF7*/*KLF4* mutant
- Clear cell meningioma: *SMARCE1* mutation
- Papillary meningioma: *PBRM1* mutation/deletion
- Rhabdoid meningioma: *BAP1* mutation/deletion

Prognostic:

- *TERT* promoter mutation- grade 3
- *CDKN2A* or *CDKN2B* homozygous deletion- grade 3
- Copy number alterations associated with worse prognosis:
  - 1p loss, 6q loss, 7p loss, 10q loss
- Inactivating alterations of *PBRM1* or *BAP1* associated with poorer outcomes in some cases

Other

- Pineal parenchymal tumor of intermediate differentiation: *KBTBD4* alteration
- Desmoplastic myxoid tumor of the pineal region, *SMARCB1*-mutant: *SMARCB1* inactivating alteration
- Choroid plexus carcinoma- *TP53* mutation (50%)- consider germline *TP53* mutation (40% have Li-Fraumeni)
- Adamantinomatous craniopharyngioma: *CTNNB1* mutation
- Papillary craniopharyngioma: *BRAF* mutation
- Pituitary blastoma- *DICER1* alteration (consider *DICER1* germline testing)

Germline considerations

- Li-Fraumeni- *TP53* germline:
  - Medulloblastoma, SHH-activated, *TP53*-mutant
    - *MYCN*/*GLI2* amplification frequent
  - Diffuse astrocytic gliomas
    - Frequent *NF1*, *MYCN* amplification
    - IDH-mutant astrocytoma (R132C/R132S)
  - Choroid plexus carcinomas
- DICER1 syndrome:
  - Embryonal tumor with multilayered rosettes
  - Pituitary blastoma
  - Primary intracranial sarcoma
- Neurofibromatosis type 1- germline *NF1* alterations
  - Neurofibroma (particularly plexiform neurofibroma), ANNUBP, MPNST
- Neurofibromatosis type 2- germline *NF2* alterations
  - Bilateral vestibular schwannomas
  - Spinal ependymoma
  - Meningioma
  - Hybrid neurofibroma/ schwannoma
- Schwannomatosis: *SMARCB1*/*LZTR1* germline mutations
  - Multiple schwannomas/less commonly meningiomas
- Von-Hippel Lindau syndrome: germline *VHL*
  - Hemangioblastoma, endolymphatic sac tumor
- Tuberous sclerosis syndrome: germline *TSC1*/*TSC2*
  - Subependymal giant cell astrocytoma
  - Subependymal nodule
  - Cortical dysplasia
- Melanoma/astrocytoma syndrome: germline *CDKN2A* alterations
  - Cutaneous melanoma, diffuse astrocytoma/PXA
- Constitutional mismatch repair deficiency syndrome (CMMRD):
  - Biallelic germline mutations of *MLH1*/*PMS2*/*MSH2*/*MSH6*
  - Ultramutated gliomas, embryonal tumors
  - May be microsatellite stable
- Lynch syndrome:
  - Heterozygous mutation of *MLH1*/*PMS2*/*MSH2*/*MSH6* or promoter methylation of *MLH1*
  - Older presentation than CMMRD, typically non-CNS
  - Typically, microsatellite high