WCN 2015, Teaching course NEURO-ONCOLOGY TC 27

Role of histology in the diagnosis of brain tumors

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Disclosure slide

Nothing to disclose

Purpose of laboratory-based brain tumor diagnosis (= tumor typing)

- Separation of clinically meaningful brain tumor entities
  (Tumor Classification)

- Assessment of biological tumor behaviour
  (Tumor Grading)

- Prediction of patient prognosis
  (Prognostic Biomarkers)

- Prediction of a patient's response to therapy
  (Predictive Biomarkers)

Laboratory tools used for brain tumor diagnosis

- Neuroimaging
- Serology (e.g. in germ cell tumors)
- Histology
- Molecular typing
- Epigenetic profiling
- Liquid biopsy

Brain tumor classification, key points

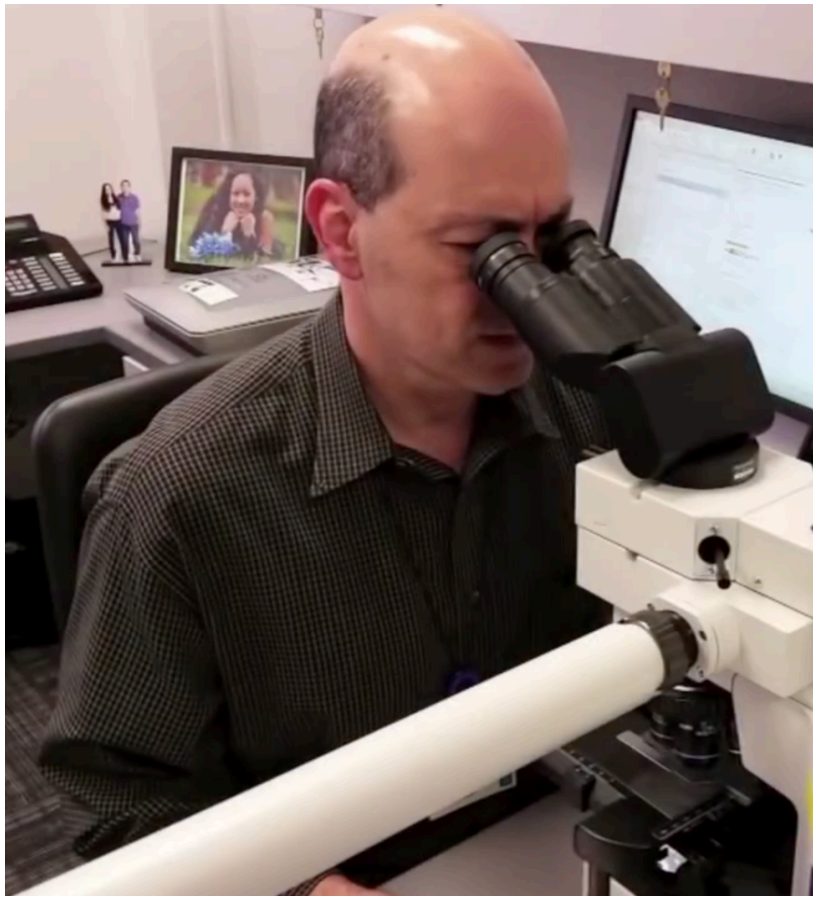
(Separation of clinically meaningful brain tumor entities)

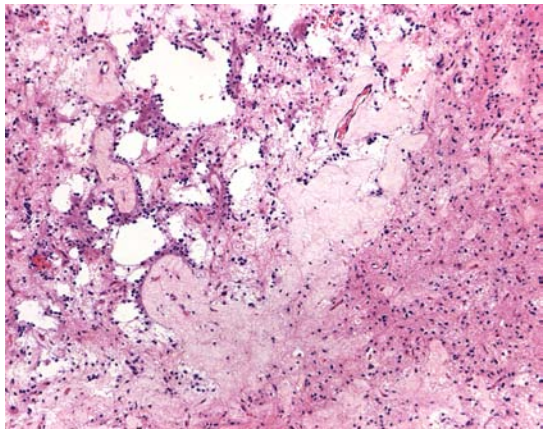
Since ca. 100 years, brain tumor classification is based on light microscopy of HE-stained sections

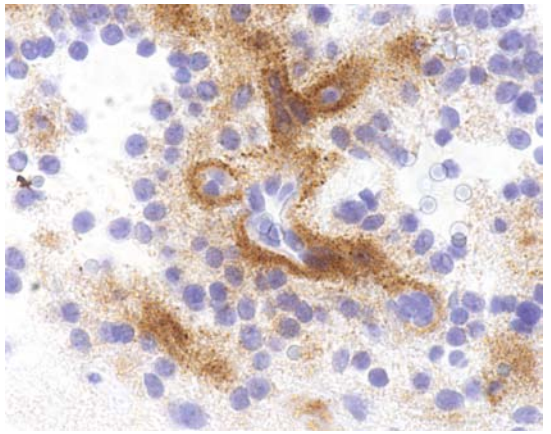
Since ca. 40 years, ancillary immunohistochemistry is in use

Histological pattern recognition by trained neuropathologists based on similarities with putative cells of origin and their developmental differentiation states

Classical approach to brain tumor classification: pattern recognition by expert neuropathologists







Brain tumor classification, key points

(Separation of clinically meaningful brain tumor entities)

Since 1979 international consensus-based criteria for brain tumor typing have been elaborated in the frame of the World health organization (WHO)

More than 100 distinct brain tumor entities have been defined on a consensus basis over time

Neuroimaging, diagnostic biomarkers, and epigenetic profiling may support brain tumor classification

The next WHO classification system will integrate histological pattern recognition with molecular markers

Evolution of the WHO brain tumor classification

Histology-only classifications

1st Edition 1979

2nd Edition 1993

3rd Edition 2000

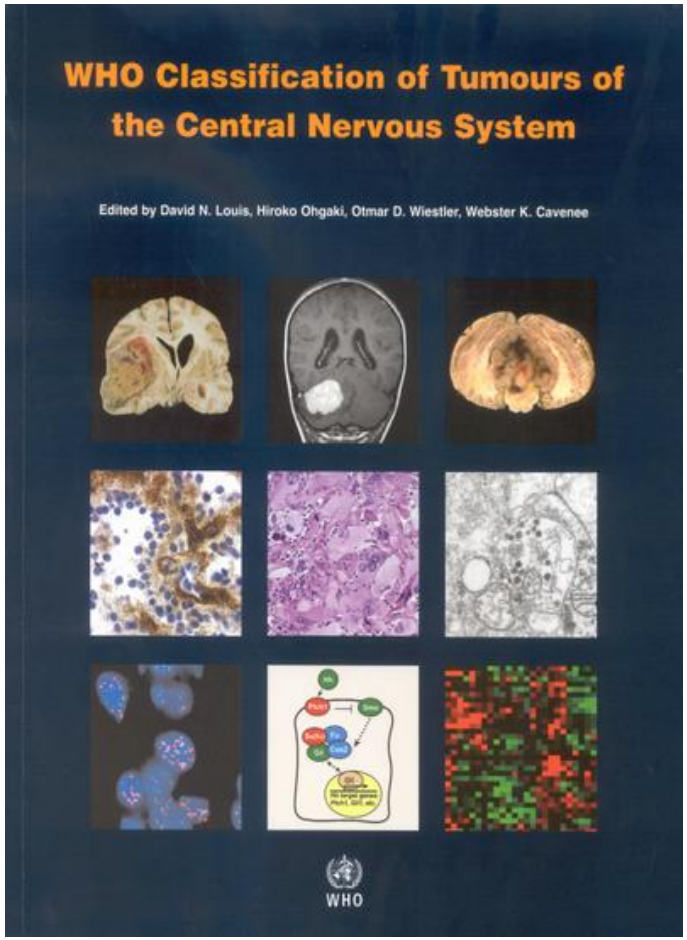
4th Edition 2007

Histology + molecular paramter-based classification

4th Edition Update 2016

Printed book + web-based publication

(www.pubcan.org)



WHO classification of tumors of the CNS

TUMOURS OF NEUROEPITHELIAL TISSUE	
Astrocytic tumours	
Pilocytic astrocytoma	9421/1[1]
Pilomyxoid astrocytoma	*9425/3**
Subependymal giant cell astrocytoma	9384/1
Pleomorphic xanthoastrocytoma	9424/3
Diffuse astrocytoma	9400/3
Fibrillary astrocytoma	9420/3
Gemistocytic astrocytoma	9411/3
Protoplasmic astrocytoma	9410/3
Anaplastic astrocytoma	9401/3
Glioblastoma	9440/3
Giant cell glioblastoma	9441/3
Gliosarcoma	9442/3
Gliomatosis cerebri	9381/3
Oligodendroglial tumours	
Oligodendroglioma	9450/3
Anaplastic oligodendroglioma	9451/3
Oligoastrocytic tumours	
Oligoastrocytoma	9382/3
Anaplastic oligoastrocytoma	9382/3

2007 scheme

Diffuse astrocytic and oligodendroglial tumours

9400/3 Diffuse astrocytoma, IDH-mutant

9411/3 Gemistocytic astrocytoma, IDH-mutant

Diffuse astrocytoma, IDH-wildtype

9400/3 Diffuse astrocytoma, NOS

9401/3 Anaplastic astrocytoma, IDH-mutant

Anaplastic astrocytoma, IDH-wildtype

9401/3 Anaplastic astrocytoma, NOS

9440/3 Glioblastoma, IDH-wildtype

9441/3 Giant cell glioblastoma

9442/3 Gliosarcoma

9440/3 Epithelioid glioblastoma

9445/3 Glioblastoma, IDH-mutant

9440/3 Glioblastoma, NOS

Diffuse midline glioma, H3 K27M–mutant

2016 update

Changes and anticipated effects of the new integrated WHO classification system

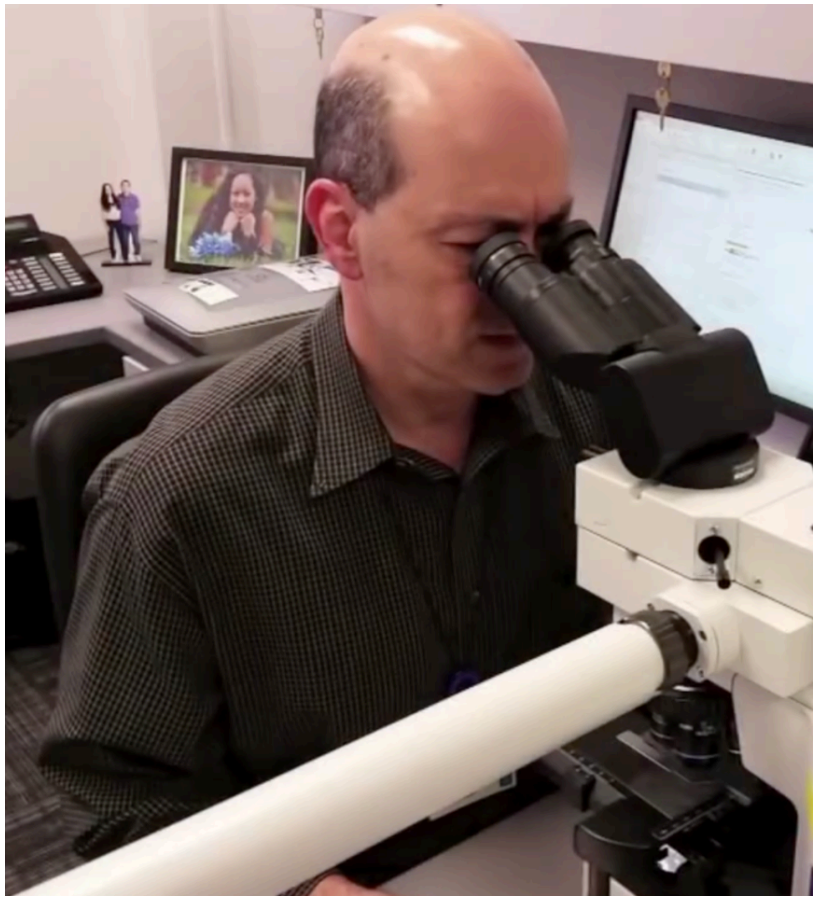
Changes in the list of tumor types: e.g. descriptive variants like fibrillary astrocytoma or protoplasmic astrocytoma will disappear from the list, instead combined histology-molecular terms will come up, e.g. diffuse astrocytoma, IDH-mutant or diffuse astrocytoma, IDH-wildtype

The term „not otherwise specified (NOS)“ will be introduced, e.g. diffuse astrocytoma, NOS

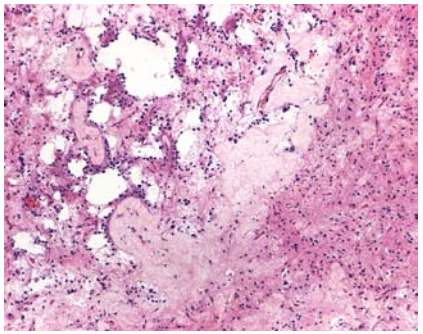
New tumor entities will be introduced, e.g. diffuse midline glioma, H3 K27M-mutant

Diagnostic Neuropathology is challenged towards setup of diagnostic molecular testing

Histology + molecular integrated brain tumor diagnostics



HE



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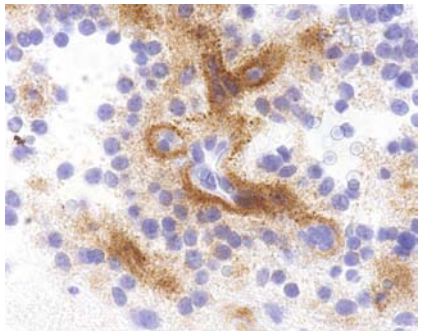
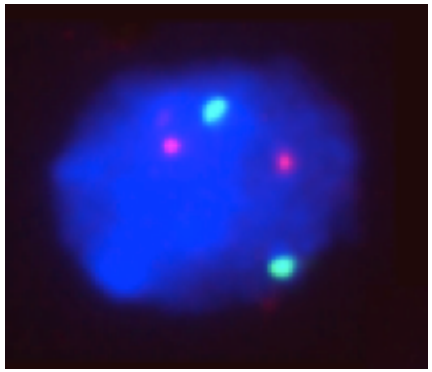
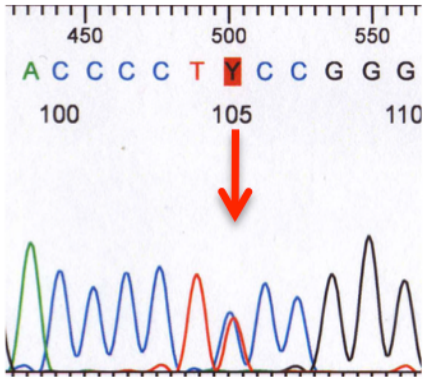


FISH



Genotyping

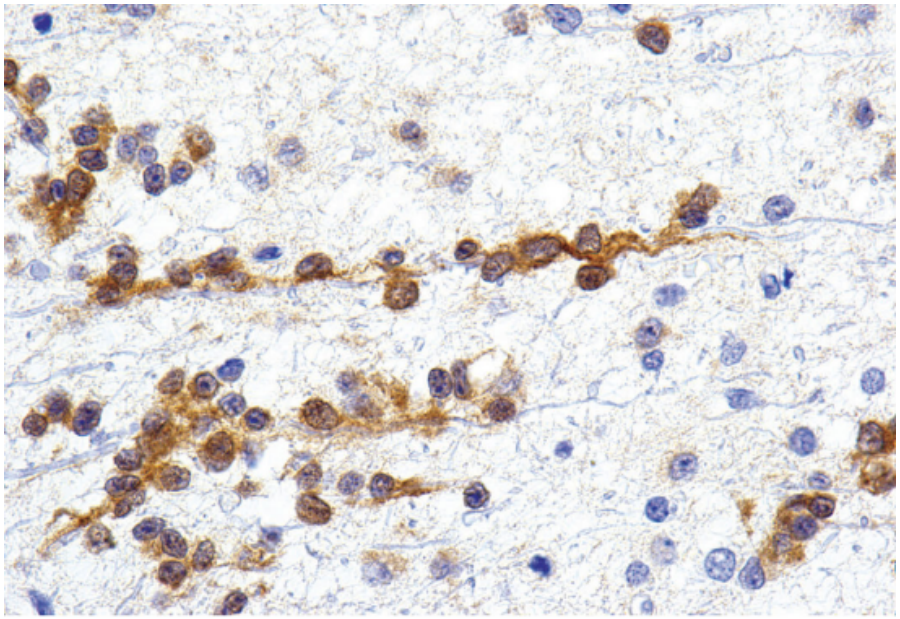


Molecular testing of brain tumors

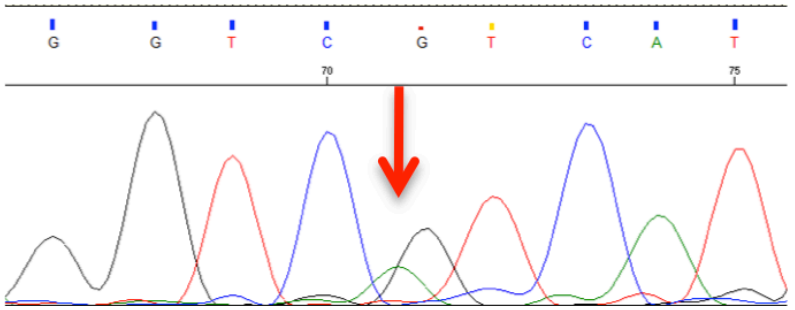
NOTA BENE

The WHO classification does no specify the type of testing required

Example given: two alternatives for IDH R132H mutation testing

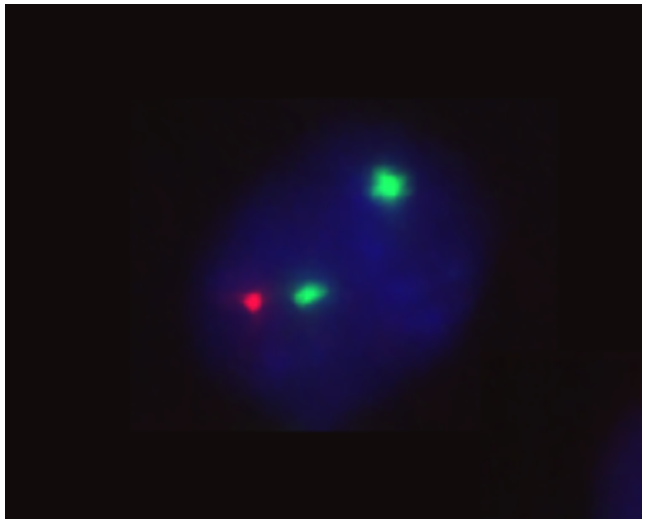


Mutation-specific anti-IDH1 antibody

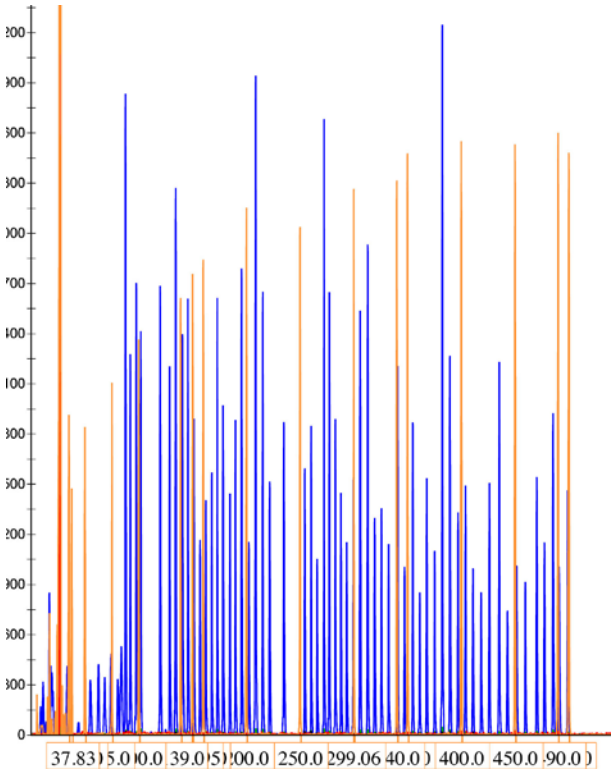


DNA- sequence analysis

Example given: two alternative methods for 1p/19q codeletion testing

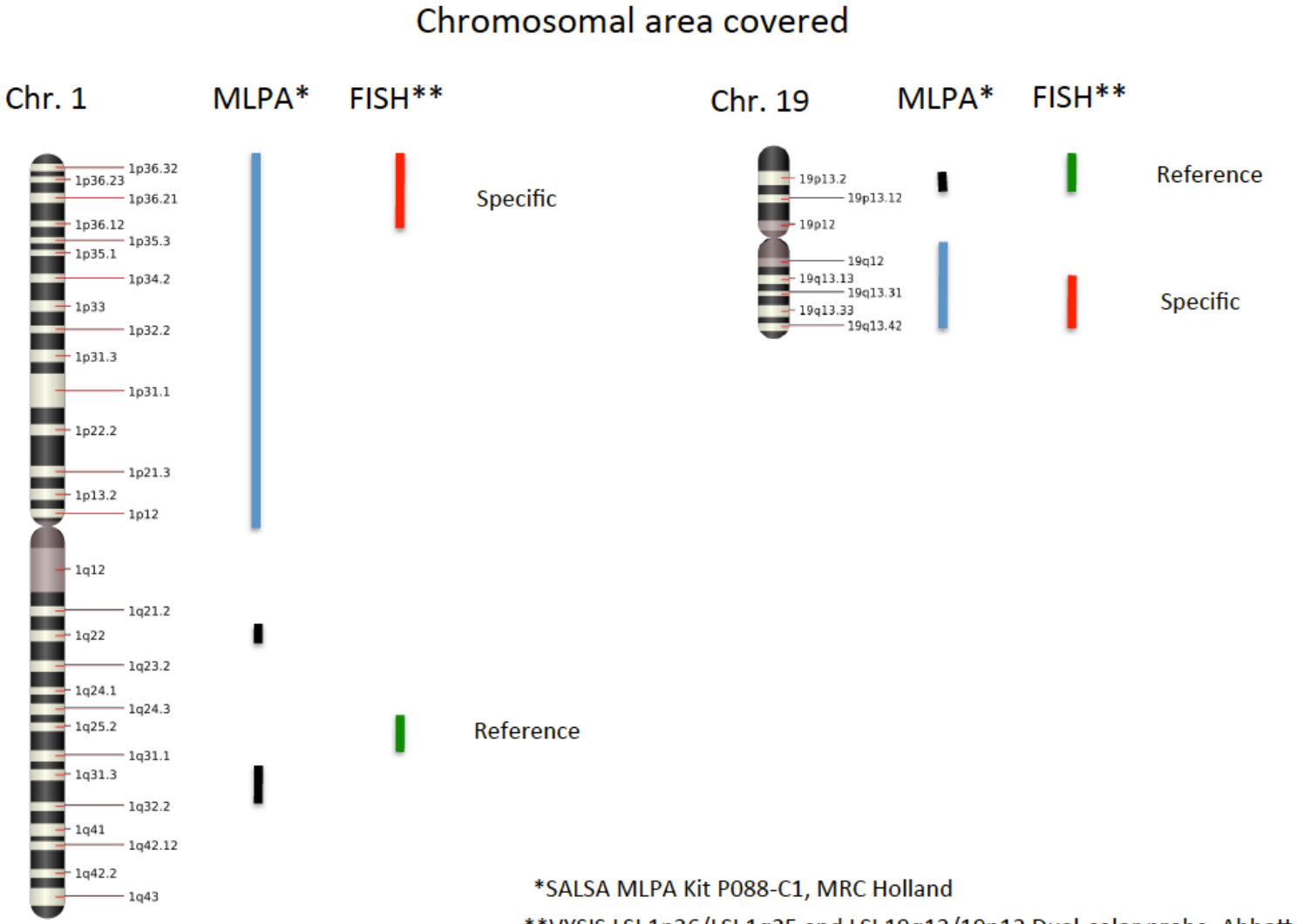


Fluorescence in situ hybridization (FISH)



Multiplex ligation-dependent probe amplification (MLPA) - analysis

1p/19q testing by MLPA and FISH - comparison



*SALSA MLPA Kit P088-C1, MRC Holland

**VYSIS LSI 1p36/LSI 1q25 and LSI 19q13/19p13 Dual-color probe, Abbott

Molecular testing of brain tumors

NOTA BENE

Non-uniform laboratory testing will lead to some degree to interlab-differences of test results

Brain tumor classification

Interlude

Not only histological patterns and molecular alterations are relevant for brain tumor typing, but also neuroimaging may be decisive for brain tumor classification

Example given:

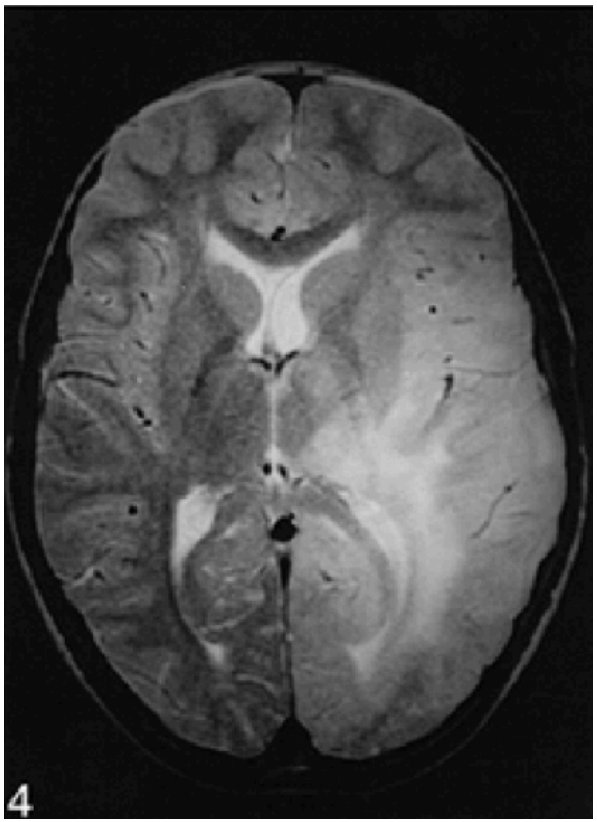
Gliomatosis cerebri

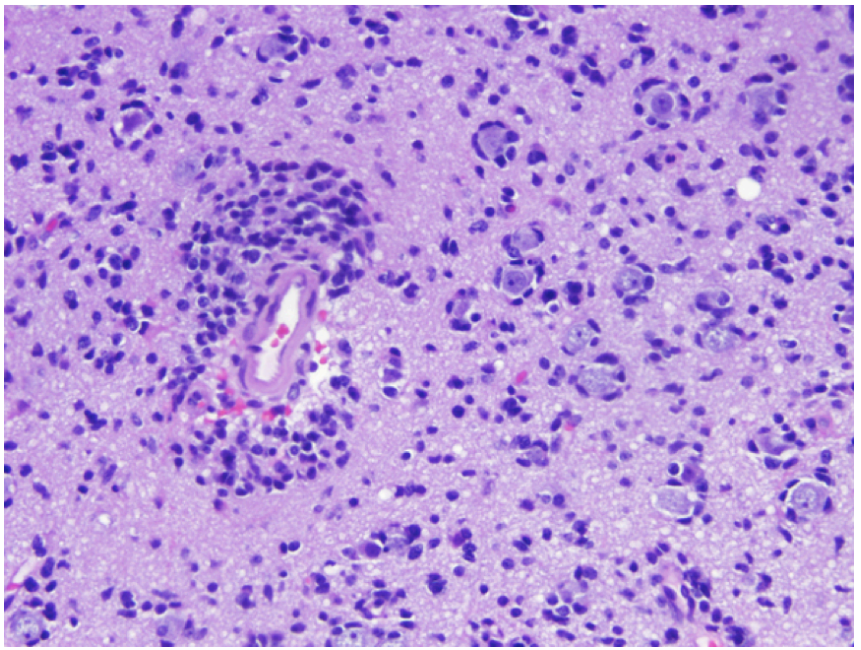
Gliomatosis cerebri - definition

A diffuse glial tumor which infiltrates the brain extensively, involving three or more lobes with diffuse enlargement of anatomic structures.

Source: www.pubcan.org

Gliomatosis cerebri: diagnosis is based on integrative consideration of neuroradiological and histological features





Whole genome epigenetic profiling
450K methylation array analysis

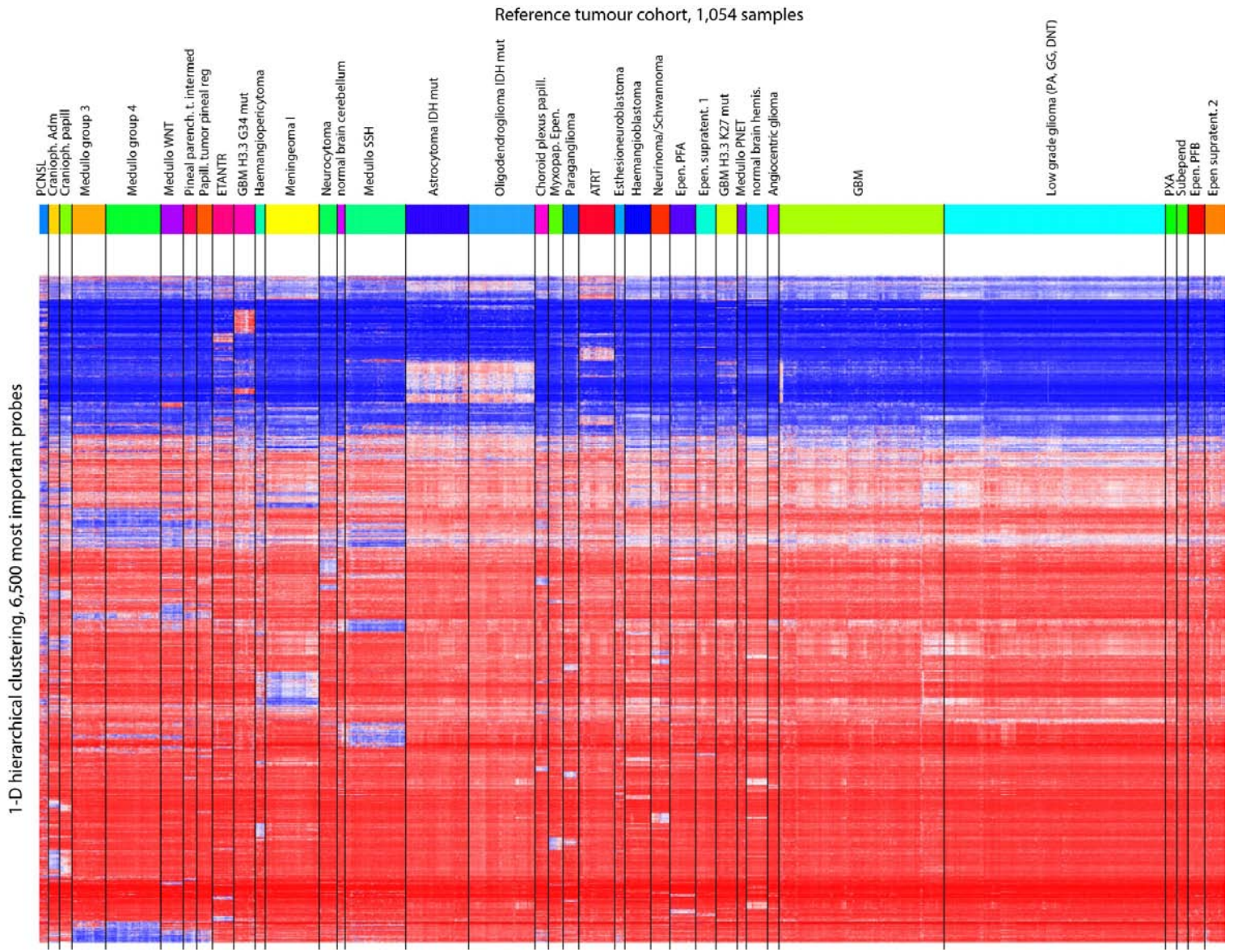
May provide complementary data for accurate brain tumor classification

Illumina Infinium HumanMethylation450 (450K) Beadarray

- single CpG site resolution
- more than 480,000 probes
- more than 99% of RefSeq-annotated genes covered
- distributed across CpG islands, 5’ and 3’ UTRs and gene bodies

- Input material: 500ng bisulfite converted DNA form FFPE or frozen tissue

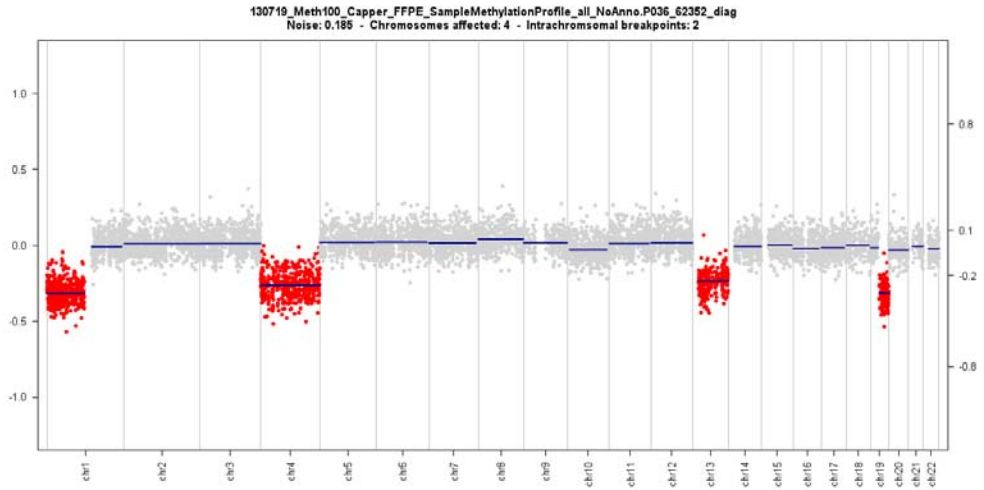
Bibikova et al., Genomics (2011)



Courtesy of Andreas vonDeimling and David Capper, Heidelberg

Example given:
Case xxx, histological diagnosis: anaplastic oligodendroglioma

Tumor ID 62352	Classifier score
Astrozytom IDH mut	0,25
Angiozent. Gliom	0,00
AT/RT	0,00
Craniopharyngeom adam.	0,00
Craniopharyngeom papill.	0,00
Esthesioneuroblastom	0,00
Ependymom Prog. Gruppe A	0,00
Ependymom Prog. Gruppe B	0,00
Ependymom Supratentoriell 1	0,00
Ependymom Supratentoriell 2	0,00
ETANTR (ETMR)	0,00
Glioblastom	0,01
Glioblastom H3.3 G34 mut	0,00
Glioblastom H3.3 K27 mut	0,01
Hämangioblastom	0,00
Hämangioperizytom	0,00
Low grade Gliom (PA, GG, DNT)	0,00
Medulloblastom Gruppe 3	0,00
Medulloblastom Gruppe 4	0,00
Medulloblastom SHH	0,00
Medulloblastom WNT	0,00
Medullo PNET	0,01
Meningeom	0,00
Myxopapilläres Ependymom	0,00
Neurozytom	0,00
Oligodendrogliom IDH mut	0,65
Plexus Papillom	0,00
Primäres ZNS Lymphom	0,00
Pinealis Parenchymtumor interm. Diff.	0,00
PXA	0,01
Paragangliom	0,00
Papill. Tumor der Pinealis Region	0,00
Subependymom	0,00
Schwannom	0,00
Normalgewebe Kleinhirn	0,00
Normalgewebe Hemisphäre	0,02



Bady et al., Acta Neuropathol (2012)

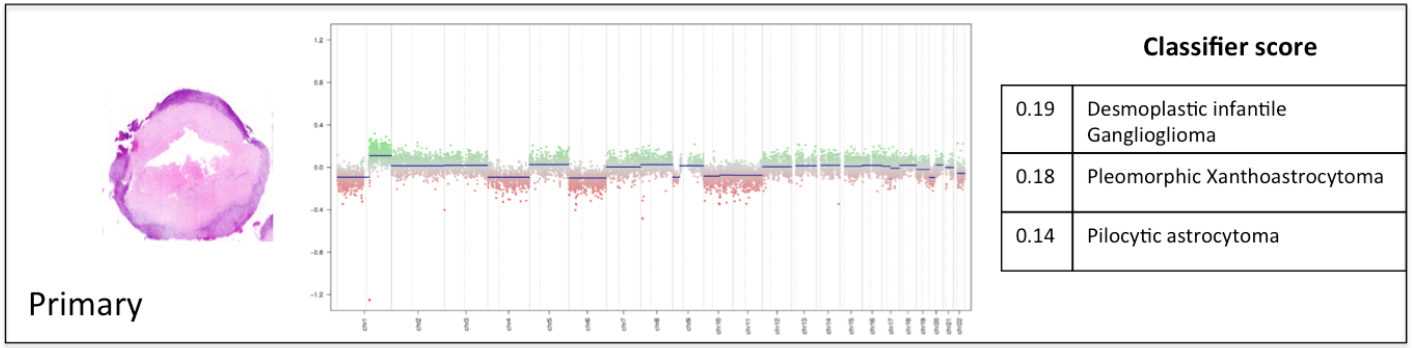
Prospect of whole genome 450K methylation profiling

- May become a valuable supplementary tool for accurate brain tumor classification, complementing histological brain tumor typing

Limitations of whole genome 450K methylation profiling

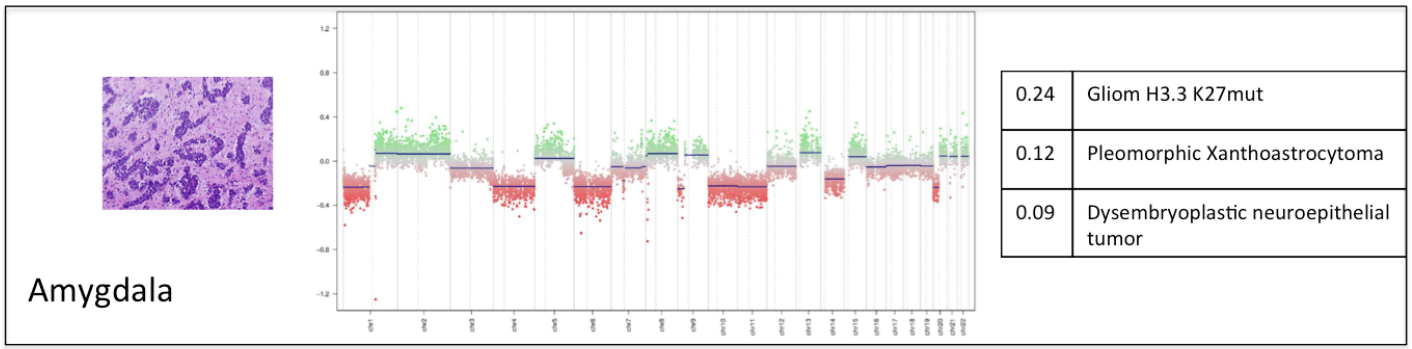
- some brain tumor cases do not show up with a significant classifier score
- brain tumors with spatially variable histological phenotypes may show up with variable classifier score profiles

Example given: methylation profiling of a disseminating brain tumor with spatial heterogeneity of phenotype yielded variable inconclusive classifier scores



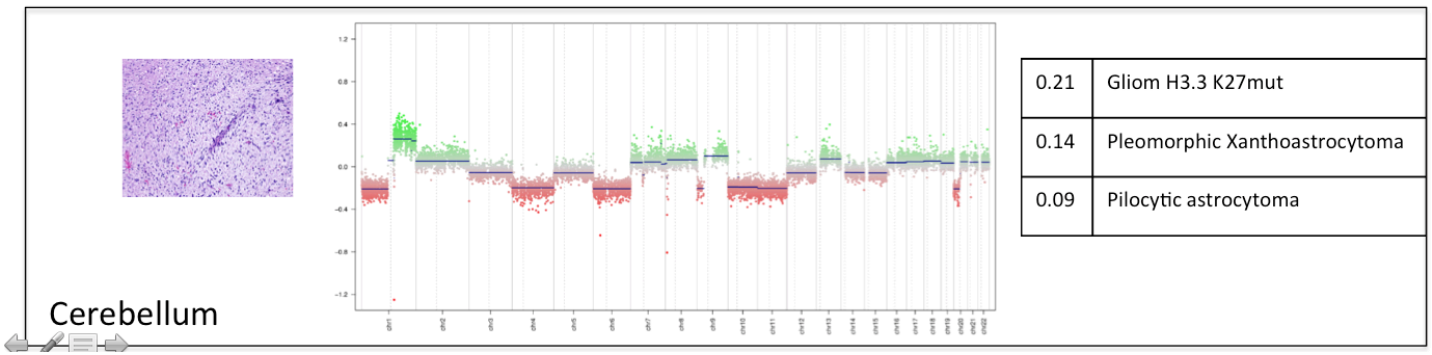
Primary

Classifier score	
0.19	Desmoplastic infantile Ganglioglioma
0.18	Pleomorphic Xanthoastrocytoma
0.14	Pilocytic astrocytoma



Amygdala

0.24	Gliom H3.3 K27mut
0.12	Pleomorphic Xanthoastrocytoma
0.09	Dysembryoplastic neuroepithelial tumor



Cerebellum

0.21	Gliom H3.3 K27mut
0.14	Pleomorphic Xanthoastrocytoma
0.09	Pilocytic astrocytoma

Brain tumor grading

(Assessment of biological tumor behaviour)

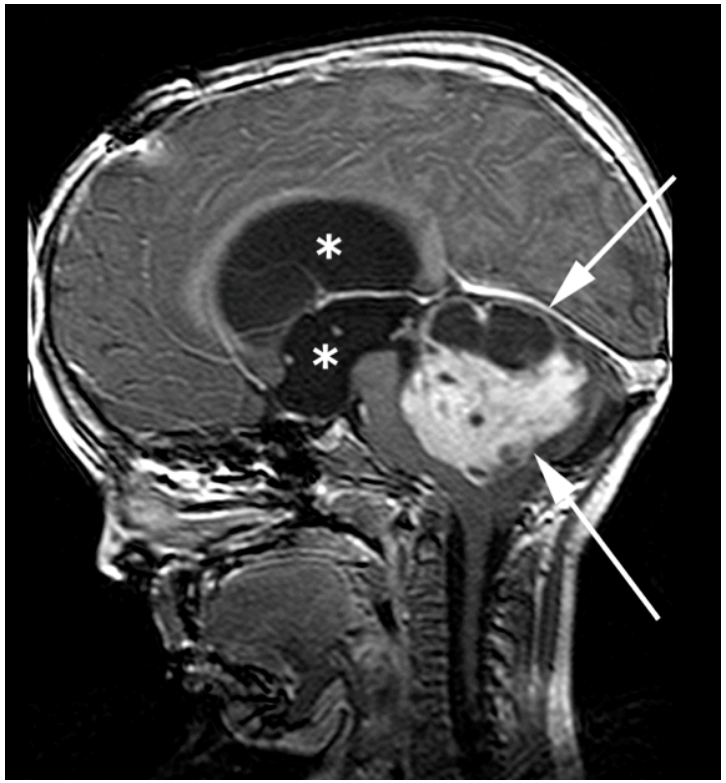
- The WHO brain tumor grading scheme is a malignancy scale across a wide variety of tumor entities rather
- WHO grading is widely used and has largely replaced previously published grading systems for brain tumors
- Grading is not an essential requirement for application of the WHO classification system
- WHO brain tumor grades have proven useful as additions to the diagnoses

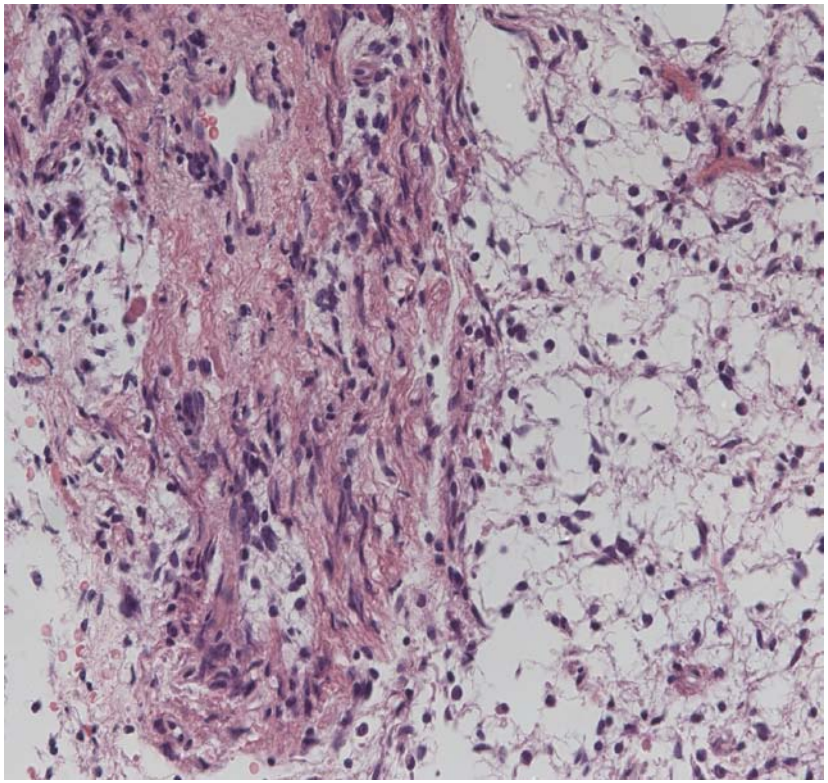
WHO brain tumor grades across entities (assessment of biological tumor behaviour)

- **WHO grade I: low proliferative potential, possibility of cure after surgical resection alone**
- **WHO grade II: usually infiltrative, low proliferation, frequent recurrence, possible progression to higher malignancy**
- **WHO grade III: histological signs of malignancy, significant proliferation, infiltrative growth, indication for postoperative radio/chemotherapy**
- **WHO grade IV: full-blown histological signs of malignancy, significant proliferation, widespread infiltration of surrounding tissue, propensity to craniospinal dissemination, rapid disease evolution**

WHO brain tumor grades across entities (assessment of biological tumor behaviour)

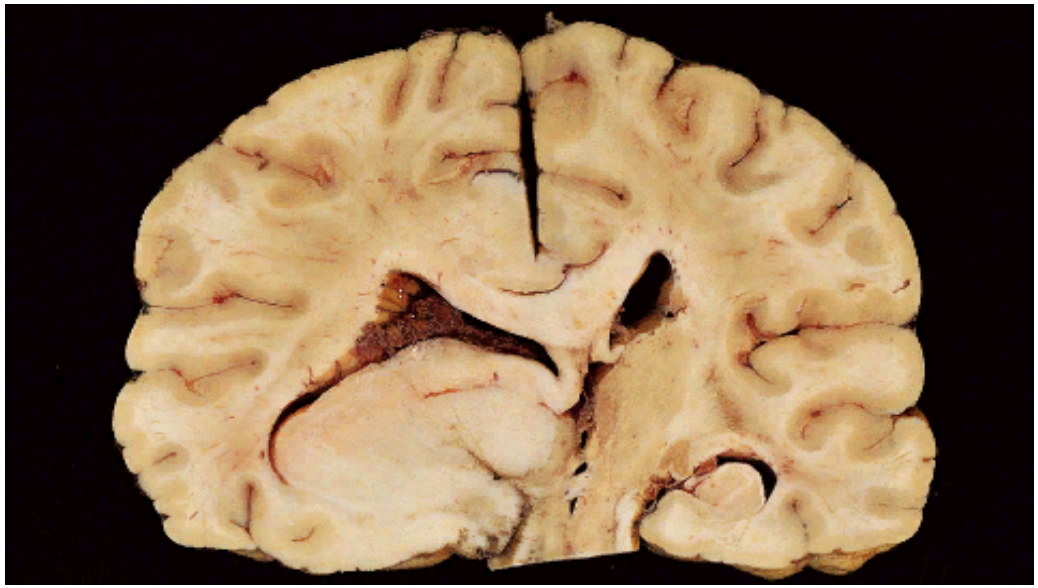
WHO grade I: low proliferative potential, possibility of cure after surgical resection alone

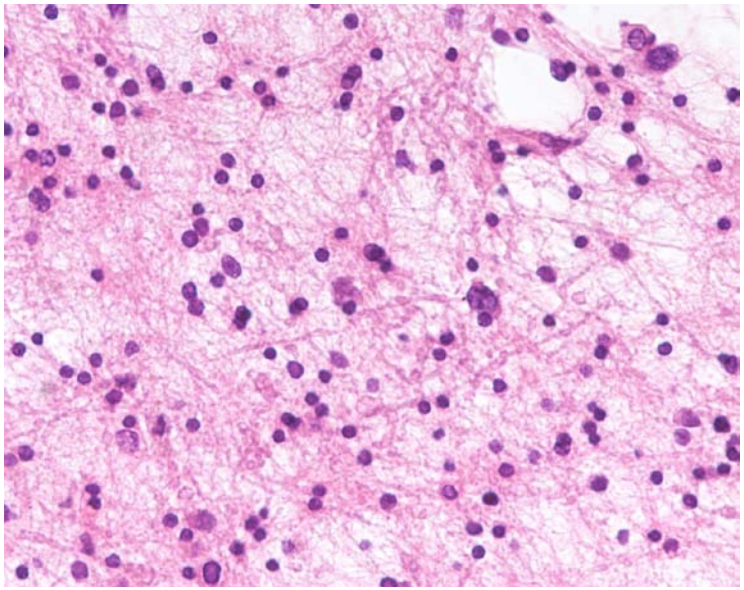




WHO brain tumor grades across entities (assessment of biological tumor behaviour)

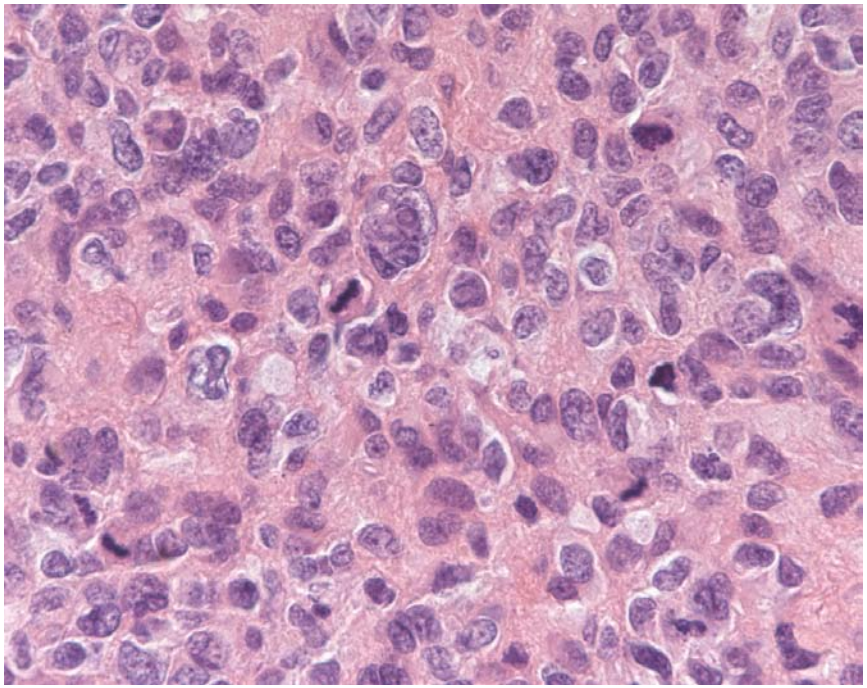
WHO grade II: usually infiltrative, low proliferation, frequent recurrence, possible progression to higher malignancy

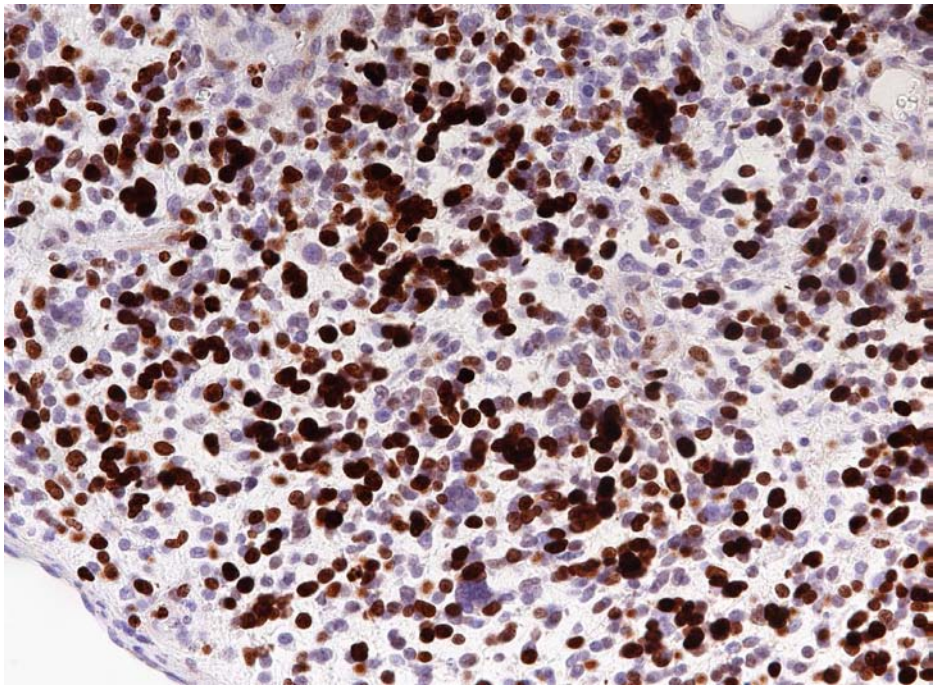




WHO brain tumor grades across entities (assessment of biological tumor behaviour)

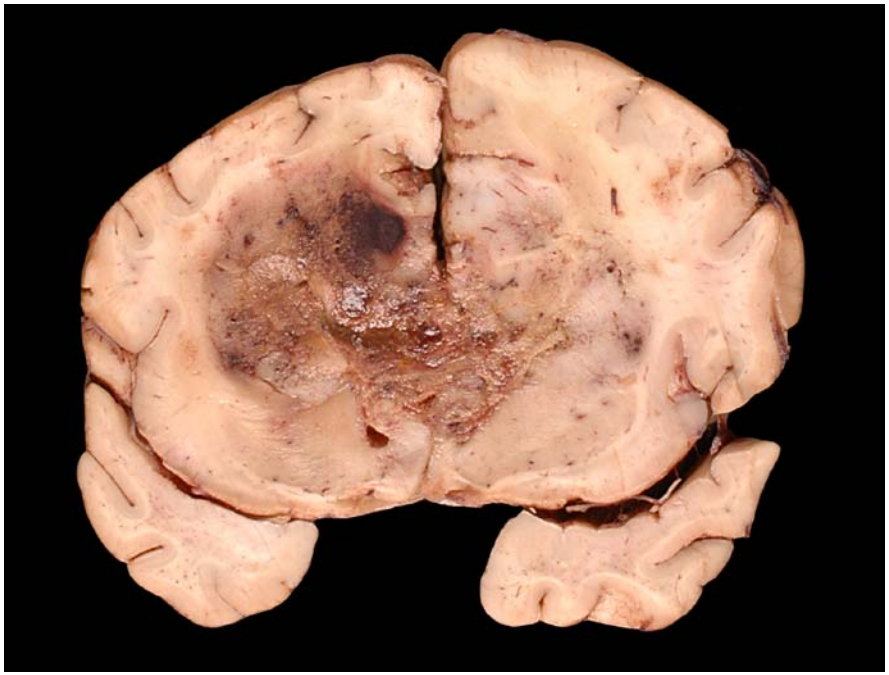
WHO grade III: histological signs of malignancy, significant proliferation, infiltrative growth, indication for postoperative radio/chemotherapy

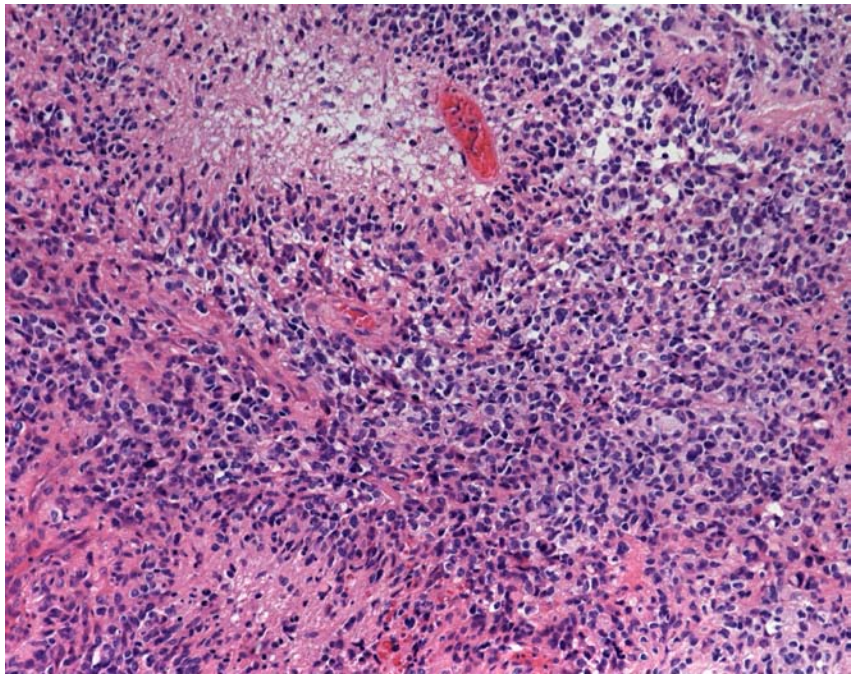




WHO brain tumor grades across entities (assessment of biological tumor behaviour)

WHO grade IV: full-blown histological signs of malignancy, significant proliferation, widespread infiltration of surrounding tissue, propensity to craniospinal dissemination, rapid disease evolution





WHO grading is used as

- Malignancy scale indicating biological behaviour of a given tumor (e.g. circumscribed versus infiltrative tumor)
- Prognostic factor indicating patient outcome and survival
- Key factor for therapeutic stratification (patients with WHO grade III brain tumors usually receive postoperative radio/chemotherapy)

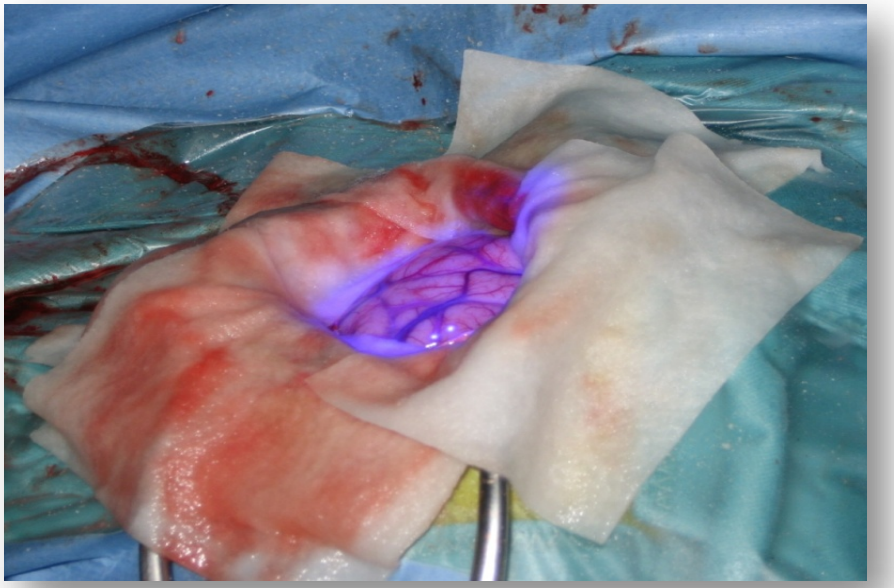
Nota bene

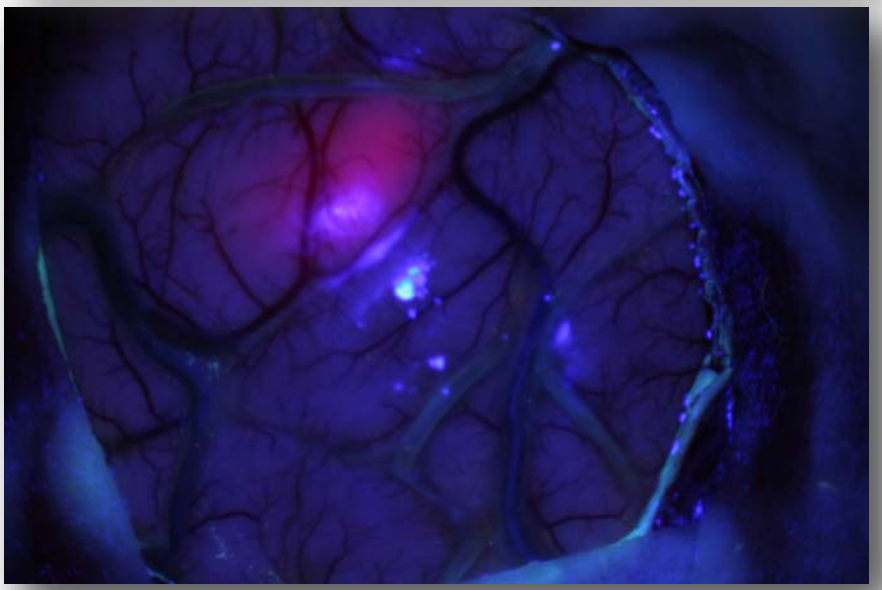
In diffuse gliomas, identification of the most malignant tumor parts in diffusely infiltrative gliomas is crucial for optimal postoperative therapy decisions

Multimodal imaging and 5-aminolevulinic acid (5-ALA) fluorescence-guided neurosurgery may optimize adequate brain tumor sampling

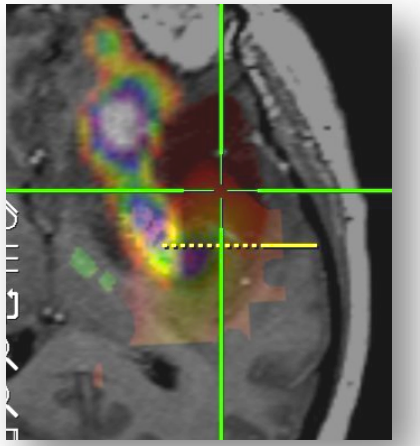
5-ALA

- 5-Aminolevulinic acid (5-ALA) leads after oral application to intracellular accumulation of **strongly fluorescing protoporphyrin IX** in malignant glioma tissue
- Fluorescence can be visualised by a **modified neurosurgical microscope** with violet-blue excitation light
- Intraoperative **identification** of **(residual)-malignant glioma tissue**



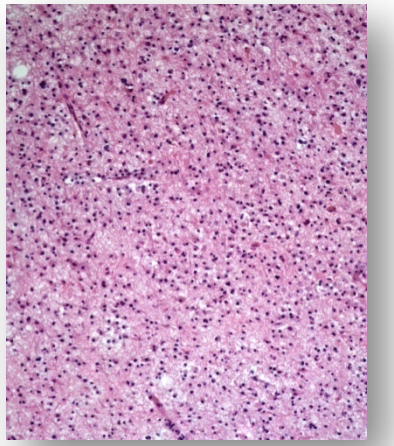


Courtesy of Stefan Wolfsberger, Department of Neurosurgery, MedUni Wien

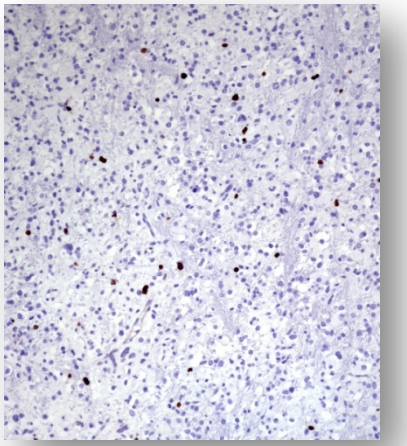


CSI + PET min

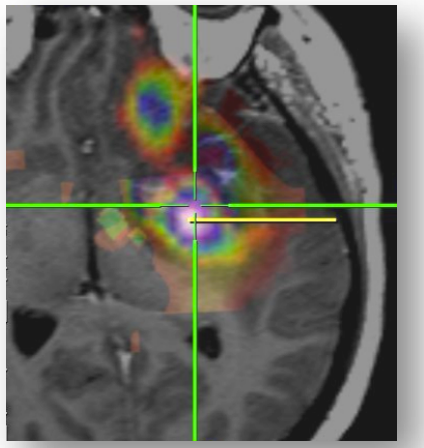
ALA -



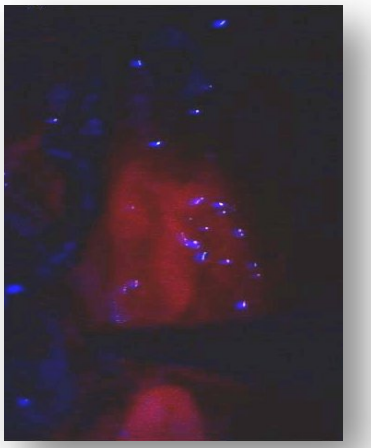
low grade tumor



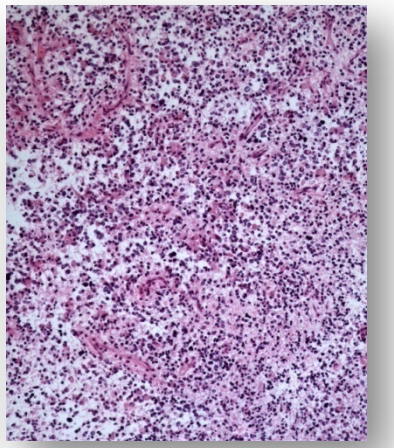
MIB-1: 5%



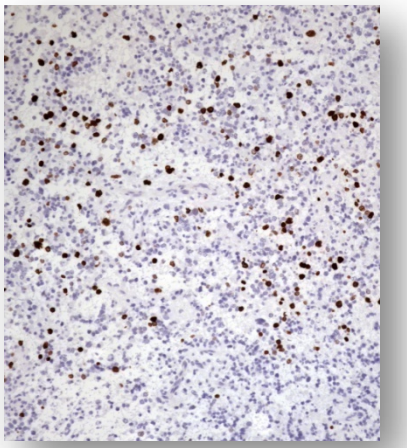
CSI + PET max



ALA +



anaplastic focus



MIB-1: 14%

Diffuse glioma with low-grade parts and an anaplastic focus

Courtesy of Stefan Wolfsberger, Department of Neurosurgery, MedUni Wien

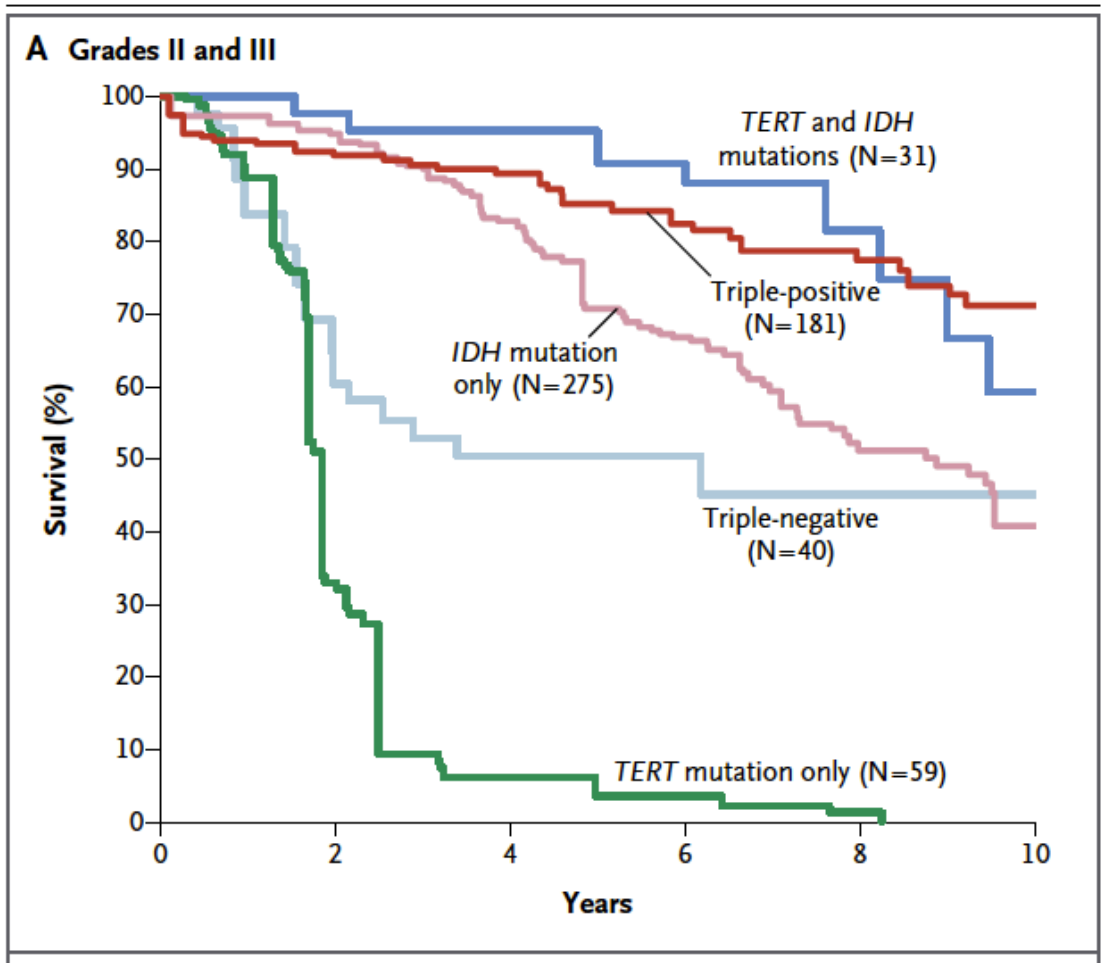
Refinement of brain tumor patient outcome prediction (prognosis) by means of molecular markers

Two recent studies have shown independently that diffuse gliomas can be categorized into prognostic groups on the basis of three tumor markers:

- **Mutations in the *TERT* promoter**
- **Mutations in *IDH***
- **Codeletions of chromosome arms 1p and 19q**

Jeanette E. Eckel-Passow et al, New England Journal of Medicine 2015

Glioma grouping based on 1p/19q, *IDH*, and *TERT* Promoter Mutations



Jeanette E. Eckel-Passow et al, New England Journal of Medicine 2015

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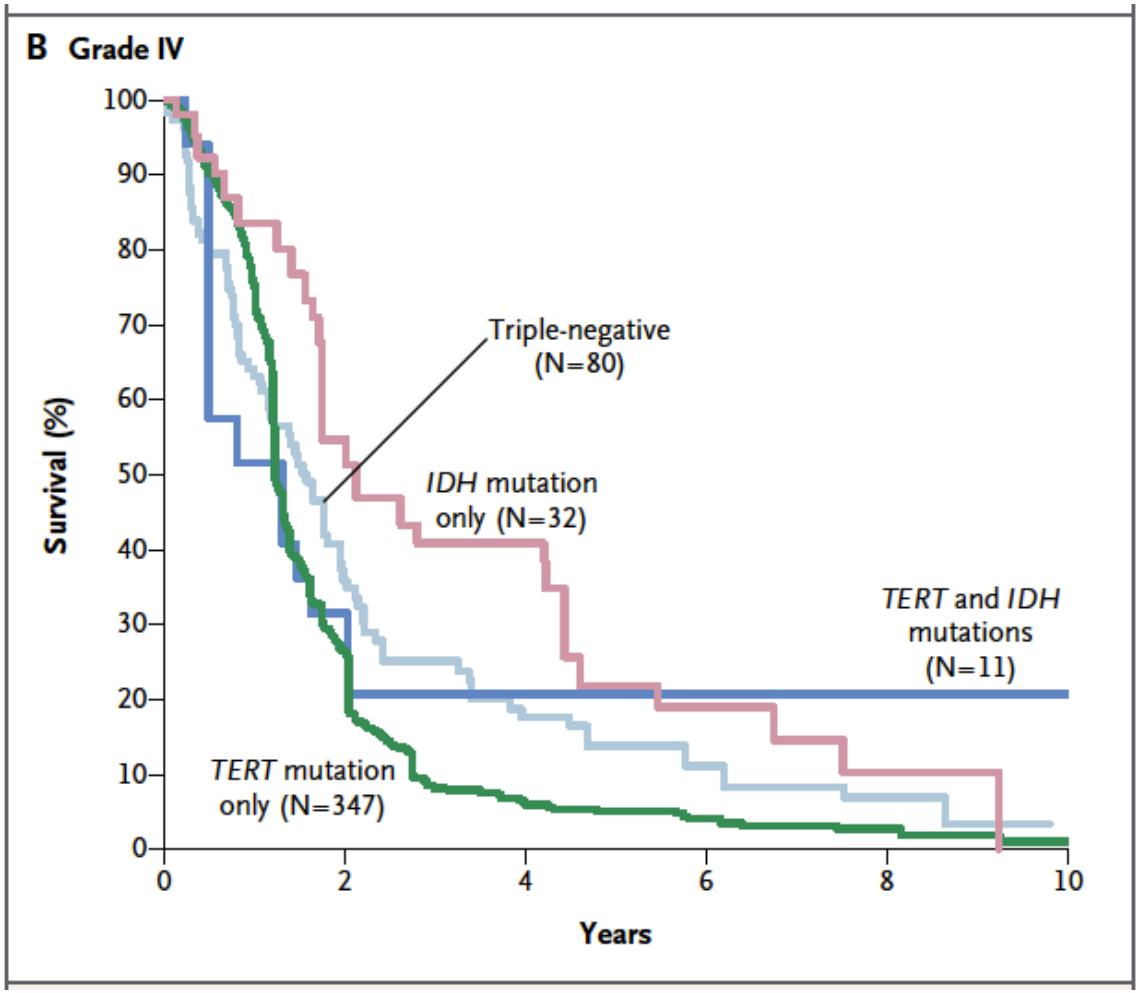
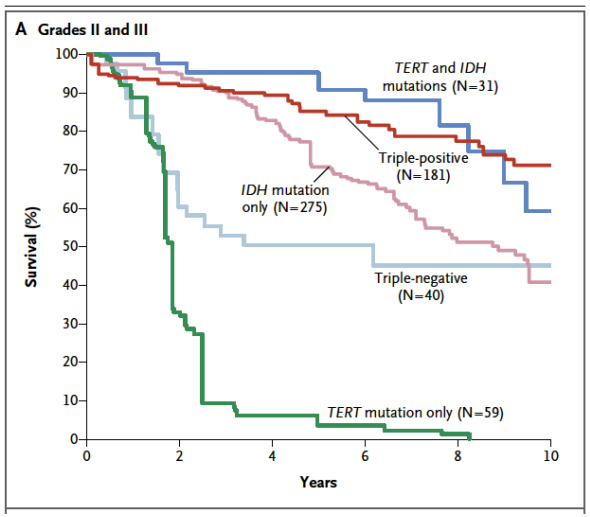
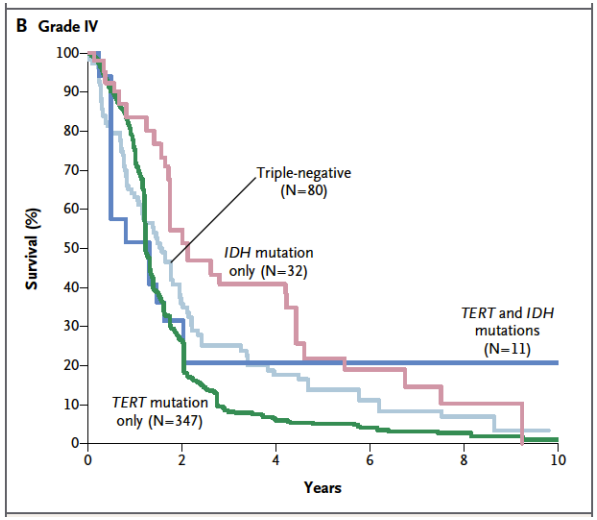
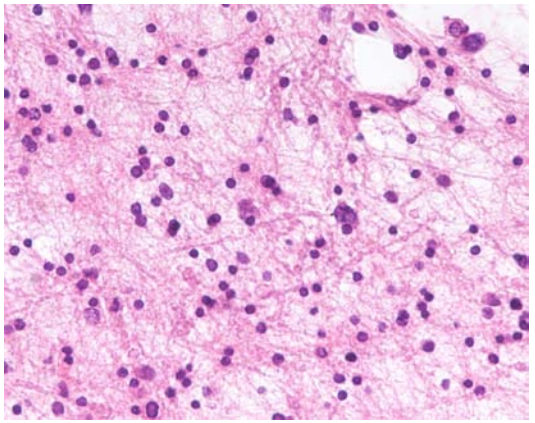


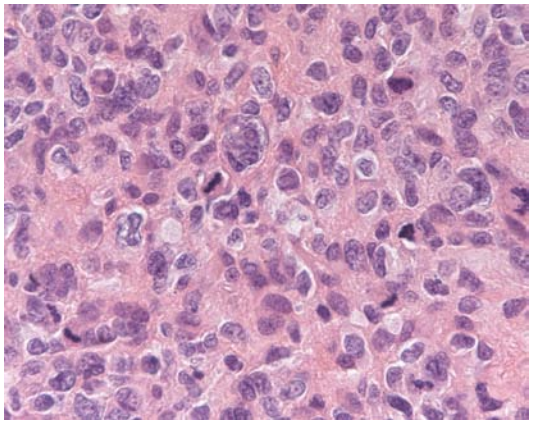
Jeanette E. Eckel-Passow et al, New England Journal of Medicine 2015

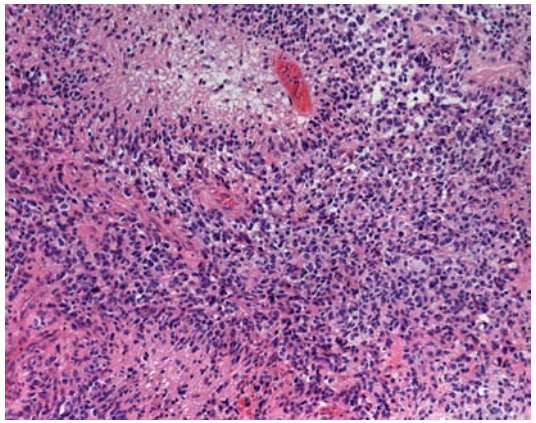
1p/19q, *IDH*, and *TERT* Promoter mutation- based glioma grouping may help to refine prognostic accuracy across tumor grades











Role of histology in the diagnosis of brain tumors
Summary and conclusions

Histology remains of key-importance in the diagnosis of brain tumors

Molecular markers will be incorporated in the next update of the WHO classification system

Whole genome epigenetic profiling will complement and refine accuracy of histology-based brain tumor classification

Molecular glioma grouping can refine prognostic accuracy across tumor grades

Altogether, appropriate integration of histology and molecular parameters harbors a high potential to advance brain tumor diagnostics towards precision medicine

Recommended reading

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) (2007) WHO Classification of Tumours of the Central Nervous System. IARC Press: Lyon

The Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. DOI 10.1056/NEJMoa1402121.

Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. N Engl J Med. DOI: 10.1056/ NEJMoa1407279.

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