DIFFUSE GLIOMAS – DIAGNOSTIC APPROACH AND ANCILLARY TESTS FOR CLASSIFICATION

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Disclosures

• I have no relevant financial relationships to disclose
Learning Objectives

• Outline the main molecular alterations in adult and pediatric diffuse gliomas
• Select immunohistochemical and molecular tests required for accurate classification of diffuse gliomas
• Classify the diffuse gliomas based on the results of the immunohistochemical stains
Classification of CNS Tumors

cIMPACT-NOW Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy–Not Official WHO
Work-up of diffuse gliomas

• Age, Location and Imaging
• Confirm that it is a diffuse glioma (IHC if needed)
• IDH1 R132H, ATRX, p53, Ki-67
• H3 K27M, H3K27me3, H3G34R/V
• Molecular tests
**DIAGNOSIS**

**Histologic features**
- Oligodendrogloma
- Oligoastrocytoma
- Astrocytoma

**IDH1/IDH2 status**
- IDH-mutant
- IDH-wildtype

**1p/19q status**
- 1p/19q-codeleted
  - Oligodendrogloma

**Histologic grade**
- Grades 2 and 3

**WHO 2016**

- (Anaplastic) Oligodendrogloma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
Case 1: 39-year-old woman with headaches

Coronal T2-FLAIR

Coronal T1-Contrast
Adult, hemispheric, enhancement (-/+)

- Low-grade histology
- IDH1 R132H (+), ATRX-intact, p53 (-/rarely+)
- 1p/19q codeletion + (FISH, aCGH, NGS, methylation)

Oligodendroglioma, IDH-mutant and 1p/19q co-deleted
Oligodendroglioma, IDH mutant & 1p/19q codeleted, WHO grade II
Diffuse glioma

Histologic features

IDH1/IDH2 status

IDH-mutant

IDH-wildtype

1p/19q status

1p/19q-codeleted

1p/19q-Intact

Histologic grade

Grades 2 and 3

(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)

DIAGNOSIS
DIAGNOSIS

Frequent alterations

IDH1/IDH2 status

1p/19q status

Histologic features

Histologic grade

1p/19q-codeleted

1p/19q-Intact

IDH-mutant

IDH-wildtype

Grades 2 and 3

Grade 4

1p/19q-Intact

Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)

Astrocytoma, IDH-mutant, WHO grade II (or III)

Glioblastoma, IDH-mutant, WHO grade IV


cIMPACT-NOW #2
Case 2: 34-year-old man with visual field cuts
Adult, hemispheric, enhancement (-/+)

- Low-grade histology
- IDH1 R132H (+), ATRX-loss, p53 (+)

Diffuse astrocytoma, IDH-mutant, WHO grade II
**DIAGNOSIS**

**Frequent alterations**
- 1p/19q status
  - 1p/19q-codeleted
  - 1p/19q-Intact

**Histologic features**
- IDH1/IDH2 status
  - IDH-mutant
  - IDH-wildtype

**Histologic grade**
- Grades 2 and 3
- Grade 4

**DIAGNOSIS**
- (Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
- (Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)
- Glioblastoma, IDH-mutant, WHO grade IV
Case 3: 37-year-old man with recurrence

Axial T1-contrast

Coronal T2 FLAIR
Anaplastic Astrocytoma, IDH-wildtype???
Adult, hemispheric, enhancement (+/-)

- Lower-grade histology (or high-grade)
- IDH1 R132H (-), ATRX-loss, p53 (+)
- IDH1/2 sequencing (or UCSF500 NGS)
**IDH1** Sanger sequencing

**IDH1 R132G**
c.C394G

Anaplastic astrocytoma, IDH mutant, WHO Grade III
<table>
<thead>
<tr>
<th>Histologic features</th>
<th>Diffuse glioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1/IDH2 status</td>
<td></td>
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<tr>
<td>1p/19q status</td>
<td></td>
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<tr>
<td>Frequent alterations</td>
<td></td>
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<tr>
<td>Histologic grade</td>
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</tbody>
</table>

### DIAGNOSIS

| (Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III) | (Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III) | Glioblastoma, IDH-mutant, WHO grade IV | Glioblastoma, IDH-wildtype, WHO grade IV |
| (Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III) | (Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III) | Glioblastoma, IDH-mutant, WHO grade IV | Glioblastoma, IDH-wildtype, WHO grade IV |

- **IDH-mutant**
  - 1p/19q-codeleted
  - 1p/19q-Intact
  - ATRX-mutant
  - TP53-mutant

- **IDH-wildtype**
  - Astrociteoma

- **Grade 2 and 3**
  - Grades 2 and 3
  - Grades 2 and 3

- **Grade 4**
  - Grade 4
  - Grade 4

- **TERT promoter mutation**
  - EGFR amplification
  - Polysomy 7 / Monosomy 10
Case 4: 62-year-old man with seizure
Older adult, hemispheric, enhancing

- High-grade histology
- IDH1 R132H (-), ATRX-intact, p53 (+/-)
  - Glioblastoma, likely IDH-wildtype; WHO grade IV
- Expect TERTp, EGFR ampl, Polysomy 7/ monosomy 10
- Others: PTEN loss, NF1, SETD2, etc...
- MGMT promoter methylation testing
- Should we do IDH1/2 sequencing?
  - Likelihood less than 1%
DIAGNOSIS

Histologic features

IDH1/IDH2 status

1p/19q status

Frequent alterations

Histologic grade

(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)

(Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)

Glioblastoma, IDH-mutant, WHO grade IV

Glioblastoma, IDH-wildtype, WHO grade IV

(Anaplastic) Astrocytoma, IDH-wildtype, WHO grade II (or III)
Diffuse Astrocytoma, IDH-wildtype

- Provisional entity in 2016 WHO
- It just says what is absent, not what is present
- Mix bag of tumors
- Significant subset behaves aggressively
Diffuse Astrocytoma, IDH-wildtype

• Provisional entity in 2016 WHO
• It just says what is absent, not what is present
• Mix bag of tumors
• Significant subset behaves aggressively
• What are the molecular alterations in these tumors correlating with worse clinical outcomes?
TERT promoter mutation → poor prognosis

Among lower grade (II-III) gliomas

and EGFR amplification → poor prognosis

Copy number alterations in diffuse gliomas

DIAGNOSIS

Frequent alterations

**IDH1/IDH2 status**

- IDH-mutant
- IDH-wildtype

**1p/19q status**

- 1p/19q-codeleted
- 1p/19q-Intact

**Histologic features**

- Diffuse glioma

**Histologic grade**

- Grades 2 and 3
- Grade 4

**Alterations associated with molecular grading**

- ATRX-mutant
- TP53-mutant
- TERT promoter mutation
- EGFR amplification
- Polysomy 7 / Monosomy 10

**DIAGNOSIS**

- (Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
- (Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)
- Glioblastoma, IDH-mutant, WHO grade IV
- Glioblastoma, IDH-wildtype, WHO grade IV
- (Anaplastic) Astrocytoma, IDH-wildtype, WHO grade II (or III)

Case 5: 57-year-old woman with new-onset seizures
Older adult, hemispheric, enhancing

- Low-grade histology
- IDH1 R132H (-), ATRX-intact, p53 (+/-)

  Diffuse astrocytoma, IDH-wildtype, WHO grade II

???
Older adult, hemispheric, enhancing

- **Low-grade histology**
- IDH1 R132H (-), ATRX-intact, p53 (+/-)
  
  Diffuse astrocytic glioma, likely IDH-wildtype; see comment

  (Molecular) glioblastoma, IDH wildtype, grade IV

- TERTp or EGFR ampl or Polysomy 7/monosomy 10
- UCSF500 next generation sequencing
TERT Promoter mutation

chr 5 1,295,228 G>A
EGFR Amplification
## PATHOGENIC AND LIKELY PATHOGENIC ALTERATIONS

<table>
<thead>
<tr>
<th>VARIANT</th>
<th>TRANSCRIPT ID</th>
<th>CLASSIFICATION</th>
<th>READS</th>
<th>MUTANT ALLELE FREQUENCY</th>
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<td>TERT upstream chr 5: g.1,295,228 G&gt;A</td>
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<td>Pathogenic</td>
<td>165</td>
<td>15%</td>
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<td>PTPRD p.D884N</td>
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<td>Likely pathogenic</td>
<td>479</td>
<td>40%</td>
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Glioblastoma, IDH-wildtype, WHO grade IV
Caveat: These markers are not 100% specific

Sensitivity and specificity for 544 IDHwt GBM in a series of 2417 brain tumors

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<thead>
<tr>
<th></th>
<th>Single</th>
<th>Double</th>
<th>Triple</th>
<th>Any double/triple</th>
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<td></td>
<td>TERT</td>
<td>EGFR</td>
<td>7/10</td>
<td>7/10 EGFR</td>
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<td>True positive</td>
<td>363</td>
<td>196</td>
<td>323</td>
<td>29</td>
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<tr>
<td>True negative</td>
<td>1674</td>
<td>1870</td>
<td>1835</td>
<td>1872</td>
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<tr>
<td>False positive</td>
<td>199</td>
<td>3</td>
<td>38</td>
<td>1</td>
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<tr>
<td>False negative</td>
<td>181</td>
<td>348</td>
<td>221</td>
<td>515</td>
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<tr>
<td>Sensitivity</td>
<td>66.7%</td>
<td>36.0%</td>
<td>59.4%</td>
<td>5.3%</td>
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<tr>
<td>Specificity</td>
<td><strong>89.4%</strong></td>
<td><strong>99.8%</strong></td>
<td><strong>98.0%</strong></td>
<td><strong>99.9%</strong></td>
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Caveat: How aggressive is molecular GBM

**DIAGNOSIS**

**Histologic features**
- Diffuse glioma

**IDH1/IDH2 status**
- IDH-mutant
- IDH-wildtype

**1p/19q status**
- 1p/19q-codeleted
- 1p/19q-Intact

**Frequent alterations**
- IDH-mutant
- IDH-wildtype
- ATRX-mutant
- TP53-mutant
- TERT promoter mutation
- EGFR amplification
- Polysomy 7 / Monosomy 10

**Histologic grade**
- Grades 2 and 3
- Grade 4

**Alterations associated with molecular grading**
- (Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
- (Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)
- Glioblastoma, IDH-mutant, WHO grade IV
- Glioblastoma, IDH-wildtype, WHO grade IV
- (Anaplastic) Astrocytoma, IDH-wildtype, WHO grade II (or III)
DIAGNOSIS

Frequent alterations

1p/19q status
- 1p/19q-codeleted
- 1p/19q-Intact

Histologic features
- Diffuse glioma

Histologic grade
- Grades 2 and 3
- Grade 4

Alterations associated with molecular grading
- ATRX-mutant
- TP53-mutant
- CDKN2A/B homozygous deletion
- TERT promoter mutation
- EGFR amplification
- Polysomy 7 / Monosomy 10
- None

1p/19q-codeleted
- IDH-mutant
- IDH-wildtype

1p/19q-Intact
- Anaplastic Astrocytoma, IDH-wildtype, WHO grade II (or III)
- Glioblastoma, IDH-mutant, WHO grade IV

IDH-mutant
- Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
- Anaplastic Astrocytoma, IDH-mutant, WHO grade II (or III)
- Astrocytoma, IDH-mutant, WHO grade 4 *

IDH-wildtype
- Glioblastoma, IDH-wildtype, WHO grade II (or III)

Case 2: Adult, hemispheric, enhancement (-/+)

- Lower-grade histology (or high-grade)
- **IDH1 R132H (-), ATRX-loss, p53 (+)**

**Anaplastic astrocytoma, IDH mutant, WHO Grade III**
**Targeted NGS panel**

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*Diffuse astrocytoma, IDH mutant, WHO Grade 4*
Adult, hemispheric, enhancement (-/+)

- Low-grade or high-grade histology
- IDH1 mutant, ATRX-loss, p53 (+)
  
  Diffuse astrocytoma, IDH-mutant, WHO grade (2 or 3)

- When to test for CDKN2A/B homozygous deletion to exclude grade 4?
  - imaging suggests high grade but histology lower grade
  - Increased mitoses and/or Ki-67 to raise suspicion for high grade
  - Clinical concern for high-grade or progression to high-grade
  - Or all?
Value of histologic grading for IDH-mutant astrocytomas

Value of histologic grading for IDH-mutant astrocytomatas

Progression free survival

Overall survival

P = 0.077

Value of histologic grading for IDH-mutant astrocytomas

Caveat: Majority of the studies showed a prognostic effect for grade 3 tumors only.
Caveat: CDKN2A HD may not even be the worst group

13 of 24 with CDKN2A deletion were grade 2
Caveat: Assessing homozygous deletion by FISH can be difficult with unclear cut-off values

DIAGNOSIS

Histologic features

IDH1/IDH2 status

1p/19q status

Frequent alterations

Histologic grade

Alterations associated with molecular grading

DIAGNOSIS

Other diagnoses to consider and rule out

Diffuse glioma

IDH-mutant

IDH-wildtype

1p/19q-codeleted

1p/19q-Intact

ATRX-mutant

TP53-mutant

CDKN2A/B homozygous deletion

TERT promoter mutation

EGFR amplification

Polysomy 7 / Monosomy 10

(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)

(Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)

Astrocytoma, IDH-mutant, WHO grade 4 *

Glioblastoma, IDH-wildtype, WHO grade IV

(Anaplastic) Astrocytoma, IDH-wildtype, WHO grade II (or III)

Pediatric-type gliomas, especially in younger adults

Non-infiltrating gliomas
### Diffuse glioma (pediatric type)

<table>
<thead>
<tr>
<th>Location</th>
<th>Midline</th>
<th>Hemispheric</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3 K27 status</td>
<td>H3 K27-altered</td>
<td>H3 K27-wildtype</td>
</tr>
</tbody>
</table>

**DIAGNOSIS**

Diffuse midline glioma, H3 K27M-altered, grade 4

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Case 6: 72-year-old man with altered mental status

Axial T2-FLAIR

Axial T1-contrast
Any age, midline

- Low-grade or high-grade histology
- IDH1R132H (-), ATRX (+/-), p53 (+/-)
- H3 K27M (+), H3K27me3-loss

Diffuse midline glioma, H3 K27M-mutant, WHO grade 4
Diffuse glioma (pediatric type)

Location
- Midline
  - H3 K27-altered
  - H3 K27-wildtype
- Hemispheric

H3 K27 status
- H3 K27-altered
- H3 K27-wildtype

Age group
- Infant
- Child/adolescent

H3 G34 status
- H3 G34-wildtype
- H3 G34-mutant

Additional mutations
- ATRX
- TP53

Other alterations
- NTRK, ROS, MET, ALK

DIAGNOSIS
- Diffuse midline glioma, H3 K27M-altered, grade 4
- Diffuse hemispheric glioma, H3 G34-mutant
- Infantile high-grade glioma, H3 wildtype
Case 7: 27-year-old woman

Apparent diffusion coefficient (ADC)  
Diffusion weighted Images (DWI)
Glioblastoma, IDH-wildtype, WHO grade IV
(Young) adult, hemispheric, enhancement (+/-)

- High-grade histology (but may be low-grade)
- IDH1 R132H (-), ATRX-loss, p53 (+)
- IDH1/2 sequencing

OR

- H3 G34R/V?
Diffuse hemispheric astrocytoma, H3 G34-mutant
WHO grade 4*
**Diffuse glioma (pediatric type)**

**Histologic grade**
- High-grade (3 and 4)
- Low-grade

**Location**
- Midline
- Hemispheric

**H3 K27 status**
- H3 K27-altered
- H3 K27-wildtype

**Age group**
- Infant
- Child/adolescent

**H3 G34 status**
- H3 G34-wildtype
- H3 G34-mutant

**Additional mutations**
- ATRX
- TP53
- NTRK, ROS, MET, ALK
- MYB, MYBL1, FGFR1, FGFR2

**DIAGNOSIS**
- Diffuse midline glioma, H3 K27M-altered, grade 4
- Diffuse high-grade pediatric-type glioma, H3 wildtype
- Diffuse hemispheric glioma, H3 wildtype
- Infantile high-grade glioma, H3 wildtype
- Diffuse midline glioma, H3 K27-altered, grade 4
- Diffuse glioma, ### alteration
- Diffuse glioma, Non-infiltrating gliomas

**Other diagnoses to consider and rule out**
- Adult-type diffuse gliomas
- Non-infiltrating gliomas

---

**cIMPACT-NOW #4**

Work-up of diffuse gliomas

• Age, Location and Imaging
• Confirm that it is a diffuse glioma (IHC if needed)
• IDH1 R132H, ATRX, p53, Ki-67
• H3 K27M, H3K27me3, H3G34R/V
• Molecular tests
Case 8: 47-year-old woman

https://pathology.ucsf.edu/aanp-teaching-session
https://pathpresenter.net/#/public/display?token=bb734709

Axial T2-FLAIR
Axial T1-contrast
THANK YOU...


