A clinical perspective on neuropathology and molecular genetics in brain tumors

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Low grades...

- Female, born 1976
- 1st seizure 2005, poorly controlled partial seizures
- Febr 2012: growth, rCBV 2.8. Gross total resection, 2 cm residual tumor left behind
- Histology: OD, minimal anaplastic characteristics
- Molecular features: 1p/19q loss, and IDH mutated
- How to treat?
WHO Classification of diffuse gliomas: cornerstone for further treatment decisions

- Glial progenitor cells
  - IDH1 mutation (>85%)
  - Common precursor cells
    - TP53 mutation (>65%)
      - Diffuse astrocytoma
        - EGFR amplification (~35%)
        - TP53 mutation (~30%)
        - PTEN mutation (~25%)
        - NF1 alteration (~20%)
        - LOH 10p (~70%)
        - LOH 10q (~70%)
        - Primary glioblastoma
    - Loss 1p/19q (>75%)
      - Oligodendroglioma
      - Anaplastic oligodendroglioma
      - Anaplastic astrocytoma
        - LOH 10q (>60%)
        - Secondary Glioblastoma
A case submitted for a clinical trial on grade III glioma (CATNON): eligible or not?

- EORTC Catnon trial
  - Joint European/North-American/Australian trial
- Investigates the best treatment for non-1p/19q co-deleted anaplastic = grade III glioma
- Eligible are
  - centrally confirmed grade III tumors
  - without 1p/19q co-deletion
A case submitted for a clinical trial on grade III glioma (CATNON): eligible or not?

• Case: 38 year old male, MRI: brain tumor
  – Operated in hospital X, local diagnosis: AA
  – Referred to hospital Y (participating to CATNON) review diagnosis: low grade astrocytoma
  – Eligible for CATNON?

• Submission for review for inclusion in CATNON
  – 1st reviewer: AA
  – 2nd reviewer: low grade astrocytoma

• Eligible????
Now what?

• RT
  – low grade tumor: 50.4 Gy
  – anaplastic tumor: 59.4 Gy

• Chemotherapy
  – Some advocate RT/TMZ for anaplastic tumors
  – Proven role for adjuvant treatment with PCV in low grade

• Is this just an incident?
Survival of anaplastic oligoastrocytoma

Time to Treatment Failure by Tumor Histology in NOA4

EORTC 26951: survival in AOD and AOA as diagnosed by the central reviewer
The presence of IDH mutations and 1p/19q loss distinguishes between favorable and unfavorable AOA

AOA1: IDH mut and/or 1p/19q codel.
AOA2: neither
Shortcomings of the current histopathological classification of gliomas

- Poor reproducibility of diagnosis in grade II and grade III tumors
  - Both with respect to lineage and grade
  - 25-33% of cases
- Based on morphological resemblance and clinical outcome (prognosis)
  - Not a functional approach
  - Not correlated to outcome to specific treatments

\(^1\) van den Bent Acta Neuropathol (2010) 120:297–304
Molecular analysis of brain tumors: stages in a development

• Yesterday: identifying adjuvant chemotherapy sensitive tumors
  – Gliomas

• Today: more precise classification of brain tumors
  – Glioma, medulloblastoma, pilocytic astrocytoma

• Tomorrow: targeted treatment
  – Glioblastoma, medulloblastoma
Established markers in neuro-oncology 2014

<table>
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<th>robust assay</th>
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<td>EGFR</td>
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<td>-</td>
<td>?</td>
<td>++</td>
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<tr>
<td>1p/19q codeletion</td>
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<td>+</td>
<td>++</td>
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<tr>
<td>MGMT</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+/-</td>
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<tr>
<td>IDH1</td>
<td>++</td>
<td>+</td>
<td>+?</td>
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Predictive markers for benefit to adjuvant chemotherapy:
- markers which presence are associated with outcome
- Are clinically most relevant: guide treatment decisions
- Which is the most useful?

Mellinghof, van den Bent ASCO 2011
MGMT promoter methylation

• MGMT protein key in resistance to alkylating, methylating chemotherapy
• In case of promoter methylation no expression and increased sensitivity to alkylating agents
• Predictive for effect to early temozolomide in newly diagnosed glioblastoma (Hegi et al, NEJM 2005)
  – And decreased outcome in temozolomide treated patients with MGMT unmethylated promoter (NOA4, Nordic elderly trial)
NOA-8 trial: RT versus TMZ in elderly GBM patients

- 373 elderly patients randomized between RT and temozolomide (1 one week on/one week off schedule)

- EFS in patients with MGMT promoter methylation: longer in TMZ treated patients
  - 8.4 months [95% CI 5.5-11.7] vs 4.6 mo [4.2-5.0] after RT

- MGMT unmethylated: opposite finding
  - 3.3 months [3.0-3.5] vs 4.6 months [3.7-6.3] after RT

Wick et al, NOA-8 trial
RT versus TMZ
Combined 1p/19q loss

- Translocation, typical for classical oligodendroglial tumors
- Described in 1998 as a marker of chemosensitivity
- 2012: predictive of benefit to adjuvant chemotherapy
- Major prognostic significance
EORTC 26951: Outcome (PFS) after RT and Genotype

- **1p/19q loss**
  - Median PFS: 9 mo
  - 5-yr PFS: 14%
  - HR [95% C.I.]: 0.34 [0.21-0.56]
  - p < 0.0001

- **No 1p/19q loss**
  - Median PFS: 62 mo
  - 5-yr PFS: 50%
  - HR [95% C.I.]:

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**Number of patients at risk:**

<table>
<thead>
<tr>
<th>LOH</th>
<th>No combined loss</th>
<th>1p&amp;19q loss</th>
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OS in 1p/19q co-deleted and intact patients

**Conclusion:** In 1p/19q co-deleted tumors clinically significant benefit of PCV
Mutations in IDH 1,2 gene

• Mutated in 70-80% of grade II, III glioma
  – Early event, diagnostic

• Encodes enzyme Krebs cycle
  – mutation causes metabolic alteration: 2GH production instead of α-ketoglutarate

• Induces a **CpG Island Methylated Phenotype** (CIMP)

• Major prognostic significance
  – Incl secondary glioblastoma
Four highly correlated factors with clinical relevance

1p/19q, IDH, CIMP and MGMT are associated

- Most 1p/19q codeleted tumors show IDH mutation
- IDH induces genome wide methylation

- Most IDH mutated tumors show MGMT promoter methylation
- MGMT promoter methylation alone optimal predictor for benefit to chemotherapy?

1\textsuperscript{Turcan et al, Nature}
Next WHO Classification of diffuse gliomas: incorporation of molecular features?

- **Glial progenitor cells**
  - IDH mutation (>85%)
  - CIMP (>85%)
  - TP53 mutations (>65%)
  - ATRX mutations (>70%)
  - TERT mutations (>80%)

- **Common precursor cells**
  - Diffuse astrocytoma
  - Oligodendroglioma

- **Anaplastic astrocytoma**
  - Anaplastic oligodendroglioma

- **Primary glioblastoma**
- **Secondary glioblastoma**
Towards a classification entirely based on genotype?

Glioma precursor cell

- IDH mutation
  - IDH mut
  - ATRX, TP53 mutation
    - AKA ‘astrocytoma’
      - OS: 5-10 yr
    - 1p/19q codeletion, TERT mutation
      - AKA ‘oligodendroglioma’
      - OS: 10-15 yr
  - TERT mut
    - TERT mut, EGFRAmpl, PTEN mut, LOH10
      - AKA ‘glioblastoma’
      - OS: 12-18 mo

unclassified
ATRX: alpha thalassemia mental retardation syndrome X linked

- Critical for normal telomere homeostasis
- Lesions are associated with alternative lengthening of telomeres phenotype (ALT)
- Mutations occur in 70% of AII, AIII
- Associated with IDH and TP53 mutations, but not 1p/91q loss
- Mutually exclusive with TERT mutations

Wick et al, NOA4, 2014
**TERT: telomerase reverse transcriptor promotor mutations**

- Hotspot mutations (C228T, C250T)
- Result in increased expression of telomerase
- Present in 1p/19q co-deleted tumors & in glioblastoma
- Mutually exclusive with ATRX

Sanson et al, Br J Canc 2014
The TCGA for low grade glioma: another step forward

Significantly mutated genes separated by molecular subtype

TCGA project on low grade glioma. Slide courtesy K Aldape
NEXT GENERATION TARGETED SEQUENCING APPROACH: ION TORRENT PGM

- Full flexibility to analyze hundreds of genes of choice
- Mutations, SNP analysis: copy number assessment
- 10 ng DNA input needed
Development of a targeted next generation sequencing panel for glial tumors

- **Major prognostic and diagnostic classifiers**
  - IDH1, IDH2, ATRX, TERT, 1p/19q, 10q, TP53, H3F3A

- **Potential drugable targets**
  - EGFR amplification, PTEN, NOTCH, BRAF, PKIC3

- **New discovered mutations in 1p/19q co-deleted tumors**
  - CIC, FUBP

- **Panel validated in EORTC 26951, on locally diagnosed anaplastic oligodendroglioma**
  - With central review available
Prognostic factor analysis OS

- **Multivariate analysis**
  - **TERT** (HR 6.22, 95% CI 2.75, 14.07)
  - **1p/19q loss** (HR 0.09, 95% CI 0.04, 0.19)
  - Both confirmed with bootstrapping (>60%)

- **Rec Part Analysis**
  - First node: **IDH** (HR 0.22, 95% CI 0.14, 0.35)
  - If IDH deleted:
    - 1st node: **1p/19q loss**
    - if no 2nd node: **TERT**

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**Model: 1p/19q loss / no 1p/19q loss & TERT wt / TERT mut (n = 113)**

**Overall Survival**

- **1p/19q loss**
- **No 1p/19q loss/TERT wt**
- **TERTmut**

**Overall Survival**

<table>
<thead>
<tr>
<th>1p/19q - TERT</th>
<th>Median (mo, 95% CI)</th>
<th>HR (95% CI)</th>
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<td>LOH 1p/19q</td>
<td>113.08 (70.87, N)</td>
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<tr>
<td>no LOH 1p/19q/TERT wt</td>
<td>33.54 (16.89, 75.40)</td>
<td>1.97 (1.10, 3.53)</td>
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<tr>
<td>no LOH/TERT mut</td>
<td>15.18 (11.43, 19.29)</td>
<td>6.52 (3.82, 11.12)</td>
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Overall survival and central path diagnosis in molecular defined glioma

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<tr>
<th>Central diagnosis</th>
<th>Missing n = 12</th>
<th>Astro n = 16</th>
<th>Oligo n = 44</th>
<th>Gliobl. n = 44</th>
<th>Total n = 116</th>
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<table>
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<th>Subtype #3</th>
<th>HR (95% CI)</th>
<th>Median (Mo) (95% CI)</th>
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<tr>
<td>Astrocytic</td>
<td>1.00</td>
<td>36.90 (16.89, N)</td>
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<td>Oligodendroglioma</td>
<td>0.58 (0.28, 1.22)</td>
<td>114.37 (70.97, N)</td>
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<tr>
<td>Glioblastoma</td>
<td>4.11 (2.03, 8.33)</td>
<td>14.62 (10.48, 19.09)</td>
</tr>
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Seizures and multiple lesions on T2 weighted imaging

- 57 year old female
- Since end of 2013 three seizures, progressive weakness left arm and sensory signs on the left
- Multiple abnormalities on T2 weighted imaging
- Dd tumor, inflammatory, mitochondrial, auto-immune disease
- No enhancement, some increase rCBV
- Decision for biopsy
Results biopsy

• **Microscopy: low grade glioma**
  – No mitosis, microvascular proliferation or necrosis
  – MIB-1 labelings-index is focally 2 a 3%

• **Targeted Next Generation Sequencing:**
  – Targets: ATRX, CIC, EGFR, FUBP1, NOTCH1, PTEN, TP53 and hotspot mutation regions in BRAF codon 600, H3F3A exon 2, IDH1 codon 132, IDH2 codon 140 en 172 en PIK3CA exon 9. Additional copy number analysis using single nucleotide polymorphism (SNP’s) of chromosome 1p, 10q, 7 en 19q.

• **Result:**
  – oncogenic mutation in PTEN-gen exon 6
  – SNP analysis: evidence for **loss of the PTEN** gene.
  – Coverage analysis: shows **EGFR gene amplification**

➢ **Inactivation PTEN, amplification EGFR: glioblastoma**
Gliomatosis cerebri/glioblastoma molecular phenotype: treatment?

- RT requires large field, almost total brain RT
- Inclination to treat with chemotherapy
- Nordic, NOA8 data: chemotherapy inferior to RT in MGMT promoter methylated tumors in elderly glioblastoma
- MS-PCR: no evidence for MGMT promoter methylation
- Decision: treatment with radiotherapy
Into the future

• Genomic wide assays allow further and improved classification of gliomas
  – Methylation arrays (epignetics)
  – Expression arrays (RNA)
  – Next generation sequencing (DNA)
• Can be run on minute quantities of (archival) FFPE material
• Allow classification, assessment of copy number alterations
Identification targets for targeted treatments: the next step

- Targeted agents aiming at very specific, molecular lesions are making their way in oncology
- No breakthrough yet in glioblastoma
- **Requires routine screening for drug-able mutations**
- Many trials targeting specific molecular alterations are already ongoing

- Ongoing trials on investigational agents target:
  - EGFRvIII mutations
  - EGFR amplification
  - PTEN mutation/loss
  - cMet amplification
  - FGFR fusion gene
Some conclusions

- Molecular factors have major prognostic implications
- Standard of care now dependant on molecular signature
- Optimal way of selecting for adjuvant chemotherapy topic of ongoing research
- Novel tools hold promise for a more functional glioma classification and for targeted treatment
But:

- Advanced platforms do not answer all issues
  - Eg, CIMP positive tumors without IDH mutations
- No platform answers all questions
  - Life is all about priorities…
- Every year a new platform…
  - RNAsequencing
- Quality control
  - Need for interlaboratory assays
Deconstructing to find a new reality?

Roy Lichtenstein. Bull series