Ki-67 and MCM6 labelling indices are correlated with overall survival in anaplastic oligodendroglioma, IDH1-mutant and 1p/19q-codeleted: a multicenter study from the French POLA network.

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INTRODUCTION

• AO IDHmut+/ 1p19qcodeletion : high grade gliomas (grade III WHO 2016)

• Prognostic factors
  • Clinical : age, Karnofsky, surgery, adjuvant radiochemotherapy
  • Histological : MV proliferation, mitotic index, necrosis, grade?
  • Molecular : $CIC$, $TCF12$, allelic loss 9p21.3
  • Machine learning : copy number variations

• Immunohistochemical :
  • Ki-67 : no clear cutoff, studies before WHO 2016
INTRODUCTION

• **MCM6**: Minichromosome Maintenance Complex component 6

  • Role in DNA synthesis, replication
  • Detectable during phases G1, S, G2 and M (absent in G0)

  • Prognostic role
    • solid tumors (NSCLC, HCC), mantle cell lymphomas
    • Meningioma, craniopharyngiomas, gliomas
OBJECTIVES

1. Evaluate/compare by immunohistochemistry the prognostic value of MCM6 and Ki-67 in AO IDHmut+/1p19qcode1 in a series from the french national multicenter POLA Network

2. Identify functional pathways dysregulated with the mRNA overexpression of these two markers using transcriptomics
MATERIAL AND METHODS

Population

• 231 cases of IDH1mut+/1p19q codel AO
  • Tissue micro-array (TMA) : 220 available
  • Centrally reviewed (WHO 2016), clinical database
• 30 cases of IDHmut+/1p19q codel oligodendrogliomas (grade II)

Ki-67 and MCM6 evaluation

• Cell counting using computerized color image analyzer
• 1-3 TMA spots/ case
• Percentage of positive nuclear stain
MATERIAL AND METHODS

• Statistics
  • Non parametrics tests
  • Overall survival (OS) $\rightarrow$ log-rank test and Cox model univariate and multivariate analyses
  • $p$-value < 0.05 = significant

• Transcriptomics
  • 68 cases from the POLA Network
  • MCM6- and MKI67-up / MCM6- and MKI67-down
  • K-means clustering, functional annotations, enrichment computations
  • Results compared with TCGA cohort
RESULTS
## Clinicopathological data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean; min-max)</td>
<td>49; 19-80 years</td>
</tr>
<tr>
<td>Sex</td>
<td>Male-to-female ratio: 1.22:1 (121/99)</td>
</tr>
<tr>
<td>Surgery</td>
<td>Biopsy: 4.1% (9/220)</td>
</tr>
<tr>
<td></td>
<td>Total resection: 32.7% (72/220)</td>
</tr>
<tr>
<td></td>
<td>Subtotal Resection 30.9% (68/220)</td>
</tr>
<tr>
<td></td>
<td>Partial Resection 20.9% (46/220)</td>
</tr>
<tr>
<td></td>
<td>Missing data 11.4% (25/220)</td>
</tr>
<tr>
<td>Type of treatment</td>
<td>RT-PCV: 32.3% (71/220)</td>
</tr>
<tr>
<td></td>
<td>Radiotherapy: 29.1% (64/220)</td>
</tr>
<tr>
<td></td>
<td>PCV: 2.3% (5/220)</td>
</tr>
<tr>
<td></td>
<td>Stupp protocol: 20.9% (46/220)</td>
</tr>
<tr>
<td></td>
<td>Temozolomide: 4.1% (9/220)</td>
</tr>
<tr>
<td></td>
<td>Other: 0.5% (1/220)</td>
</tr>
<tr>
<td></td>
<td>No treatment: 6.4% (14/220)</td>
</tr>
<tr>
<td></td>
<td>Missing data: 3.6% (8/220)</td>
</tr>
<tr>
<td>Survival</td>
<td>Progression: 30.7% (71/220)</td>
</tr>
<tr>
<td></td>
<td>Death: 8.2% (19/220)</td>
</tr>
<tr>
<td>Molecular Data</td>
<td>$TERT$ promoter mutation: 98.3% (216/220)</td>
</tr>
<tr>
<td></td>
<td>$CIC$ loss: 61% (141/220)</td>
</tr>
</tbody>
</table>

MCM6 mean LI: 24% (range 0.1-87%; median 21.4%)

Ki-67 mean LI = 6.3% (range 0.1-36.9%; median 3.7%)

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RT-PCV, radiation therapy – procarbazine, CCNU (lomustine), and vincristine.
Overall survival (Kaplan-Meier method)

**A**
MCM6 mean LI (%)
- Lesser than 50%
- Equal to or greater than 50%

**B**
Ki-67 mean LI (%)
- Lesser than 15%
- Equal to or greater than 15%

**C**
Ki-67 and/or MCM6
- Ki-67 < 15% and MCM6 < 50%
- Ki-67 ≥ 15% and/or MCM6 ≥ 50%

**Results**
- **MCM6**
  - $P = 0.013$
- **Ki-67**
  - $P = 0.001$
- **Ki-67 and/or MCM6**
  - $P = 0.004$
Univariate and multivariate analyses for OS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cox univariate (OS)</th>
<th>Cox multivariate (OS)</th>
<th>Cox multivariate (OS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR [95%CI]</td>
<td>p-value</td>
<td>HR [95%CI]</td>
</tr>
<tr>
<td>Age</td>
<td>1.055 [1.021-1.091]</td>
<td>0.001*</td>
<td>1.060 [1.020-1.103]</td>
</tr>
<tr>
<td>Mitotic index $\geq 8 / 1.6 \text{ mm}^2$</td>
<td>2.587 [1.177-5.686]</td>
<td>0.018*</td>
<td>1.439 [0.538-3.851]</td>
</tr>
<tr>
<td>MCM6 LI $\geq 50%$</td>
<td>3.283 [1.221-8.826]</td>
<td>0.018*</td>
<td>2.896 [0.964-8.702]</td>
</tr>
<tr>
<td>Ki-67 LI $\geq 15%$</td>
<td>3.948 [1.442-10.41]</td>
<td>0.008*</td>
<td>2.713 [0.935-7.875]</td>
</tr>
<tr>
<td>MCM6 LI $\geq 50%$ and/or Ki-67 LI $\geq 15%$</td>
<td>3.875 [1.603-9.370]</td>
<td>0.003*</td>
<td></td>
</tr>
</tbody>
</table>

HR, hazard ratio; LI, labelling index; OS, overall survival; * statistically significant ($P < 0.05$).
<table>
<thead>
<tr>
<th>GO biological process</th>
<th>Fold enrichment</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell cycle DNA replication initiation (GO:0102292)</td>
<td>22.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DNA strand elongation involved in cell cycle DNA replication (GO:0102296)</td>
<td>21.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ndc80 complex (GO:031262)</td>
<td>21.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pre-replicative complex assembly (GO:036388)</td>
<td>21.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Condensed nuclear chromosome kinetochore (GO:017704)</td>
<td>15.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mitotic centrosome separation (GO:017700)</td>
<td>15.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mitotic chromosome condensation (GO:017706)</td>
<td>14.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IRES-dependent translational initiation (GO:02192)</td>
<td>14.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aster (GO:05818)</td>
<td>13.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Exodeoxyribonuclease activity (GO:04529)</td>
<td>12.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Mitotic prophase (GO:088)</td>
<td>12.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Regulation of transcription involved in G1/S transition (GO:083)</td>
<td>11.9</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

FDR, false discovery rate; GO, gene ontology.

<table>
<thead>
<tr>
<th>GO biological process</th>
<th>Fold enrichment</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condensin complex (GO:05796)</td>
<td>50.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Replication fork protection complex (GO:031298)</td>
<td>42.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DNA strand elongation involved in cell cycle DNA replication (GO:0102296)</td>
<td>40.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ndc80 complex (GO:031262)</td>
<td>40.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pre-replicative complex assembly (GO:036388)</td>
<td>35.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Condensed nuclear chromosome kinetochore (GO:017704)</td>
<td>31.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MCM complex (GO:042555)</td>
<td>28.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mitotic centrosome separation (GO:017700)</td>
<td>28.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mitotic chromosome condensation (GO:017706)</td>
<td>27.5</td>
<td>&lt;0.0001</td>
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<tr>
<td>Kinetochore microtubule (GO:03828)</td>
<td>25.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aster (GO:05818)</td>
<td>25.5</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

FDR, false discovery rate; GO, gene ontology.
**A**

- MCM6 down
- MCM6 up
- MKI67 down
- MKI67 up

**B**

- c0 84.6%
- c1 99.9%
- c2 95.0%
- c3 98.1%
- c4 93.6%
- c5 95.0%
- c6 96.4%
- c7 99.9%
- c8 94.6%
- c9 97.5%

**Cluster c1 - Synaptic activity**
- Positive regulation of short / long term synaptic potentiation
- Dopamine / catecholamine / GABAergic synapse
- Oxidative stress-induced apoptotic pathway
- Corticosterone / glutamate secretion
- Axon / dendrite extension

**Cluster c2 - Glial and neural cells**
- Neuron ensheathment
- Dendritic cell differentiation / apoptotic process
- Glial cell development
- Immune response
- Water-soluble vitamin biosynthetic process
- Long-chain fatty acid metabolic process
- Glutathione metabolism

**Cluster c3 - Replication and mitotic cell cycle**
- Helicase - THO / MCM complexes
- Transcription - spliceosome
- Translation - chaperin complex
- Negative regulation of histone H3-K9 methylation
- Positive regulation of histone H3-K4 methylation
- Histone exchange
- Regulation of DNA damage
- Double-strand break repair via homologous recombination

**Cluster c7 - Cell cycle (continued)**
- Transcription - ncRNA / snoRNA
- Translation - Ribosome / ER
- G1 and G2 checkpoints / progression

**Cluster c6**
- Dicarboxylic acid catabolic process
- Nitric oxide mediated signal transduction
- Alpha amino acid metabolic process
Grades II and III IDH-mutant 1p19q codeleted oligodendrogliomas - TCGA cohort of 98 samples

A

MCM6 median expression (from grade II)

MCM6 down

MCM6 up

B

Mitotic cell cycle – replication: POLA c3+c7 (ID=73.6%)

Myelin sheath – glial cell differentiation: POLA c2 (ID=60.6%)

Mitochondria (oxphos) – methylosome: POLA c7 (ID=30.1%)

Inflamasome - positive regulation of immune response: POLA c2 (ID=48.2%)

Axoneme – oxoacid metabolic process – IL5 production: POLA c6 (ID=48.2%)

Histone modification – transcription – DNA repair: POLA c3+c7 (60.7%)

Synaptic activity – neuron development: POLA c1 (ID=53.4%)

C

Cluster1 = 6.80e-13

Cluster2 = 5.16e-09

Cluster3 = 1.01e-02

Cluster4 = 4.90e-02

Cluster5 = 4.34e-08

Cluster6 = 6.16e-02

Cluster7 = 6.64e-03
CONCLUSION

• Prognostic value of Ki-67 and MCM6 LI in AO IDHmut+/1p19q codel
  • Independently correlated to shorter survival in multivariate analyses
  • Easy-to-use and cost effective markers, could be used in routine practice
  • Could be integrated into therapeutic/clinico-radiological monitoring strategies

• Transcriptomic analyses
  • High proliferation $\rightarrow$ down-regulated immune response and lower microglial cell activation

• Article under submission, Brain Pathology (major revisions)
Thank you for your attention!

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  • POLA Network for contributing
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  • University of Lorraine