Prognostic impact of MGMT promoter methylation status in glioblastoma patients treated with Carmustine Wafer implants after surgery

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GLIOBLASTOMA (GBM)

- Incidence in the US and Europe is about 3 new cases/100,000 people/yr.
- About 16% of all primary brain tumors.
- Incidence increases with age.
- More common in older adults (age 75-84 yr).
- More common in men than women.
- About 3% of brain tumors in children.
- Less than 5% of patients survived after 5 yr post diagnosis.
“Stupp Protocol”


In 2005 adjuvant radiotherapy and concomitant oral TMZ chemotherapy were considered the standard treatment for patients with glioblastoma.

European Organization for Research and Treatment of Cancer [EORTC] protocol 26981 also known as the “Stupp protocol”.
MGMT is a DNA repair protein that excise cytotoxic methyl adducts from the O\textsuperscript{6}-guanine position in DNA, preventing mismatch and cross-linking of dsDNA.

**MGMT promoter hypermethylation → MGMT transcriptional silencing → inhibition of MGMT expression**
MGMT promoter methylation in GBM →
  inactivation MGMT gene →
  MGMT protein not expressed →
  inactivation of DNA repair →
  response to alkylating therapy
The **Gliadel wafer** is a **polyanhydride implant** used in the treatment of malignant glioma.

Gliadel containing **carmustine** (*BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea)*), a nitrosourea **alkylating agent** incorporated into a biodegradable copolymer matrix used to control the **released of carmustine over a period of 2 to 3 weeks**.

It was demonstrated that BCNU wafer has an OS benefit in newly-diagnosed and recurrent malignant glioma (*Westpal et al. 2003; Stupp et al. 2009*).
Prognostic value of MGMT in glioblastomas treated with alkylating agents

- MGMT is methylated in about 40% of GBM patients

- MGMT methylation status is a potent prognostic factor for patients who receive RT/TMZ (Hegi et al. 2005)

- patients who had methylated MGMT promoter status had significantly longer PFS and OS (Lechapt-Zalcman et al. 2012)

- MGMT promoter methylation status in GBM is a useful predictor of responsiveness to carmustine (Esteller et al. 2000)
- 82 GBM patients
- diagnosed 2007-2013
- treated with Stupp protocol and Gliadel wafer implant

<table>
<thead>
<tr>
<th>Characteristics of patients</th>
<th>No. of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>59</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>57.2 ± 8.7</td>
</tr>
<tr>
<td>Range</td>
<td>33 - 70</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>53 (64.63%)</td>
</tr>
<tr>
<td>Women</td>
<td>29 (35.37%)</td>
</tr>
<tr>
<td><strong>MGMT</strong></td>
<td></td>
</tr>
<tr>
<td>methylated</td>
<td>50 (60.98%)</td>
</tr>
<tr>
<td>unmethylated</td>
<td>32 (39.02%)</td>
</tr>
<tr>
<td><strong>IDH1/2</strong></td>
<td></td>
</tr>
<tr>
<td>mutated</td>
<td>4 (4.88%)</td>
</tr>
<tr>
<td>wild type</td>
<td>78 (95.12%)</td>
</tr>
<tr>
<td><strong>Ki-67, % positive cells</strong></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>25%</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.6% ± 15.1%</td>
</tr>
<tr>
<td>Range</td>
<td>5 % - 70%</td>
</tr>
</tbody>
</table>
MGMT promoter methylation analysis

1. Bisulfite treatment of denatured DNA
   \[ \text{mC} \rightarrow \text{mC} \]
   \[ \text{C} \rightarrow \text{U} \]

2. PCR amplification
   \[ \text{mC} \rightarrow \text{C} \]
   \[ \text{U} \rightarrow \text{T} \]

PYROSEQUENCING

DNA extraction

Sample Prep

MGMT plus®, diatech pharmacogenetics
MGMT status

60.98%  
MGMT met

39.02%  
MGMT unmet
IDH1-2 status

- IDH1 wt: 95.12%
- IDH2 wt: 100.00%
- IDH1 mut: 4.88%
- R132H
Survival Analysis

Survival Function for MGMT

Cum Survival vs Months_OS

MGMT:
- non met
- metilato

Graph showing survival function for MGMT with cumulative survival plotted against months.
CONCLUSIONS

- MGMT Methylated patients had longer OS (p=0.031)
- Ki-67 didn’t affect OS
- IDH1 mutations (R132H) didn’t affect OS
- The extent of resection significantly affect OS (p=0.001)