Biomarkers of Brain Tumors to Temozolomide Treatment

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&

« NCCR Molecular Oncology », ISREC

ENASCO Meeting, November 15-17, 2007
Predictive Factor(s) for Temozolomide (TMZ) Derived Benefit for Glioblastoma Patients

Who are the patients who benefit from TMZ?

573 patients enrolled

Concomitant TMZ/RT

Adjuvant TMZ

RT Alone

Logrank, $p < 0.0001$

<table>
<thead>
<tr>
<th></th>
<th>RT</th>
<th>TMZ/RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS, mo:</td>
<td>12.1</td>
<td>14.6</td>
</tr>
<tr>
<td>2-yr survival:</td>
<td>10%</td>
<td>26%</td>
</tr>
<tr>
<td>HR [95% CI]:</td>
<td>0.63</td>
<td>[0.52-0.75]</td>
</tr>
</tbody>
</table>

**0⁶-Methylguanine-DNA Methyltransferase (MGMT)**

- **TMZ**, alkylating agent

**MGMT**
- CHR 10q26
- linked with resistance to alkylating agent therapy
- inducible:
  - RT
  - genotoxic agents
  - glucocorticosteroids

**MOUSE MODELS & Alkylating Agents**
- MGMT-/- hypersensitive
- MGMT-Tg resistant to tumor formation

**O⁶-methylguanine**
- irreversible inactivation
- degradation

**Guanine**
- repair
Silencing of the *MGMT* Repair Gene by Methylation of the Gene Promoter

06-Methylguanine-DNA Methyltransferase (MGMT)

**Tumor**
- methylated
- « turned off »
- no expression

**Normal**
- expression

**Tumor**
- No repair protein
- No repair of TMZ treatment induced DNA damage
- Response to tumor treatment
- Improved survival of glioblastoma patient

Hegi et al_06
**MGMT** Promoter Methylation Predicts Better Outcome in Glioblastoma Patients of this Trial

<table>
<thead>
<tr>
<th></th>
<th>Unmeth</th>
<th>Meth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS, mo:</td>
<td>12.2</td>
<td>18.2</td>
</tr>
<tr>
<td>2-yr survival:</td>
<td>7.8%</td>
<td>34.1%</td>
</tr>
<tr>
<td>HR [95% CI]:</td>
<td>0.45 [0.32-0.61]</td>
<td></td>
</tr>
<tr>
<td>Logrank test:</td>
<td>$p &lt;0.0001$</td>
<td></td>
</tr>
</tbody>
</table>

Risk of death reduced by 55%

*MGMT* Promoter Methylation Predicts Benefit from TMZ Treatment

Unmethylated *MGMT*

- Randomization: RT  TMZ/RT
- Median OS mo:  11.8  12.7
- 2-yr survival:  1.9%  13.8%

Methylated *MGMT*

- Randomization: RT  TMZ/RT
- Median OS mo:  15.3  21.7
- 2-yr survival:  22.7%  46.0%

Logrank: \( p = 0.062 \)

Logrank: \( p = 0.0074 \)

Predictive Value of *MGMT* Methylation for Overall Survival

Overall Wald test: $p < 0.0001$ (df=3)

![Graph showing survival rates and treatment effects](image)

**Treatment at progression**

at discretion of treating physician

<table>
<thead>
<tr>
<th>Randomization</th>
<th>RT [%]</th>
<th>TMZ/RT [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Repeat RT</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Any Additional Chemotherapy</td>
<td>72</td>
<td>58</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>Palliative Care only</td>
<td>17</td>
<td>22</td>
</tr>
</tbody>
</table>

Stupp *et al* NEJM 2005

Progression Free Survival Supports

*MGMT* Methylation Status as Predictive Factor for Benefit from TMZ

Overall Wald test: \( p < 0.0001 \) (df=3)

<table>
<thead>
<tr>
<th></th>
<th>Unmeth, RT alone</th>
<th>Unmeth, TMZ/ RT</th>
<th>Meth, RT alone</th>
<th>Meth, TMZ/ RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients at risk:</td>
<td>28 9 0 0 0 0 0 0</td>
<td>44 18 8 8 7 5 3 1</td>
<td>44 18 8 8 7 5 3 1</td>
<td>44 18 8 8 7 5 3 1</td>
</tr>
<tr>
<td>Months</td>
<td>54 54 54 54</td>
<td>54 54 54 54</td>
<td>54 54 54 54</td>
<td>54 54 54 54</td>
</tr>
</tbody>
</table>

Hegi et al. 06
Review of the literature, August 2007
data from 1376 patients from 15 publications

MGMT Promoter Methylation Ranges from 20 to >80% Depending on Glioma Subtype

<table>
<thead>
<tr>
<th>WHO grade</th>
<th>Brain</th>
<th>1 Pilocytic</th>
<th>2 LA</th>
<th>3 AA</th>
<th>4 GB</th>
<th>4 PrGB</th>
<th>4 ScGB</th>
<th>2 O</th>
<th>3 AO</th>
<th>2 OA</th>
<th>3 AOA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>169</td>
<td>145</td>
<td>672</td>
<td>155</td>
<td>45</td>
<td>73</td>
<td>51</td>
<td>18</td>
<td>13</td>
</tr>
</tbody>
</table>

MGMT Promoter Methylation Ranges from 20 to >80% Depending on Glioma Subtype
Validation of MGMT as Predictive Factor
Depletion of MGMT in Tumor Cells by a Dose Dense Schedule

Integrated Translational Research Program:
Identification of other resistance factors and new targets

Study Chairs:
Mark R. Gilbert, M.D. (Medical Oncology)
Minesh Mehta, M.D. (Radiation Oncology)
Ken Aldape, M.D. (Neuropathology and Correlative Biology)
Arnab Chakravarti, M.D. (Neuropathology and Correlative Biology)

EORTC
Roger Stupp, M.D. (Medical Oncology)
Monika Hegi, Ph.D. (Neuropathology and Correlative Biology)
Trials in GBM based on the RT/TMZ→TMZ scheme

<table>
<thead>
<tr>
<th>Investigational Agent</th>
<th>Standard Treatment</th>
<th>Phase</th>
<th>No. of Patients</th>
<th>End Point(s)</th>
<th>Sponsor</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrovorum</td>
<td>TMZ/AFT</td>
<td>II</td>
<td>58</td>
<td>PF8</td>
<td>Marik Sevato</td>
<td>Annual randomized, multicenter (Europe), phase III trial in preparation</td>
</tr>
<tr>
<td>Citrovorum</td>
<td>TMZ/AFT</td>
<td>IIA</td>
<td>112</td>
<td>OS</td>
<td>NABTT</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>TMZ/AFT</td>
<td>I</td>
<td>46</td>
<td>Safety and toxicity</td>
<td>NCI-CTG</td>
<td>Started May 2006</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>TMZ/AFT</td>
<td>IIA</td>
<td>46</td>
<td>Feasibility, PFS, OS</td>
<td>University of Heidelberg, Germany</td>
<td>No maintenance TMZ</td>
</tr>
<tr>
<td>CDDP</td>
<td>TMZ/AFT</td>
<td>II</td>
<td>85-375</td>
<td>PF8</td>
<td>Collisson</td>
<td>Tumor-suppressing vaccine for EBV-radiation sensitive tumors</td>
</tr>
<tr>
<td>Imitinib</td>
<td>TMZ/AFT</td>
<td>IIA (randomized)</td>
<td>190</td>
<td>Survival</td>
<td>EORTC</td>
<td>Phase I completed; further drug development discontinued</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>TMZ/AFT</td>
<td>II</td>
<td>70</td>
<td>UCLA</td>
<td>Requires fresh frozen tumor tissue</td>
<td></td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>TMZ/AFT</td>
<td>III</td>
<td>508</td>
<td>Survival</td>
<td>Benalmadah</td>
<td>Start planned summer 2007</td>
</tr>
<tr>
<td>Eribulin</td>
<td>TMZ/AFT</td>
<td>IIA</td>
<td>72</td>
<td>Survival (ph1b)</td>
<td>Lilly</td>
<td>Start February 2008</td>
</tr>
<tr>
<td>Eribulin</td>
<td>XRT</td>
<td>II</td>
<td>54</td>
<td>PF8</td>
<td>Lilly Germany</td>
<td>Only for patients with an absence of prior TMZ attempts</td>
</tr>
<tr>
<td>Tiptitab</td>
<td>TMZ/AFT</td>
<td>I</td>
<td>50</td>
<td>Safety and toxicity</td>
<td>NABTC</td>
<td>Computed</td>
</tr>
<tr>
<td>Tiptitab</td>
<td>XRT</td>
<td>IIA</td>
<td>27</td>
<td>Institut Claude Regaud, University of Lausanne</td>
<td>France, Switzerland, Germany</td>
<td></td>
</tr>
<tr>
<td>Lomustine</td>
<td>XRT</td>
<td>IIA</td>
<td>30</td>
<td>Survival</td>
<td>DFCI</td>
<td>Phase 1 in phase II in combination with TMZradiation therapy planned</td>
</tr>
<tr>
<td>Vemuraminib</td>
<td>TMZ/AFT</td>
<td>IIA (randomized)</td>
<td>152</td>
<td>Survival</td>
<td>DFCI</td>
<td>Start planned summer 2007, Hartford, MSKCC, University of Virginia, Pittsburgh</td>
</tr>
<tr>
<td>Vaprelin</td>
<td>TMZ/AFT</td>
<td>II</td>
<td>41</td>
<td>PF8, survival</td>
<td>National Cancer Institute, USA</td>
<td>Vaprelin as a histone deacetylase inhibitor</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>TMZ/AFT</td>
<td>III</td>
<td>89</td>
<td>Safety, PF8</td>
<td>Novartis</td>
<td>Bioreductive drug targeting hypoxic cells</td>
</tr>
<tr>
<td>Carmustine wafers</td>
<td>TMZ/AFT</td>
<td>II</td>
<td>22</td>
<td>Survival</td>
<td>Johns Hopkins</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: TMZ, temozolomide; AFT, irradiation; NABTT, New Approaches to Brain Tumor Therapy CNS Consortium; NCI-CTG, North Central Cancer Treatment Group; EORTC, European Organization for Research and Treatment of Cancer; EUDOC, enzymes inducing antiproliferative drugs; UCLA, University of California, Los Angeles; NABTC, North American Brain Tumor Consortium; DFCI, Dana-Farber Cancer Institute; MSKCC, Memorial Sloan-Kettering Cancer Center, New York; PF8, progression-free survival; OS, overall survival; PF6, 6-month PFS rate.
Cilengitide Phase III for GBM

**Diagnosis**

- Step 1: central analysis of MGMT methylation status

- MGMT unmethyl.

  Phase I/II studies in preparation

  New Agent: to be defined

  To be defined

  Radiotherapy

- MGMT methyl.

  Step 2: Randomization

  versus Control

  + Cilengitide

  + TMZ

  Radiotherapy

**Registration**

- Randomiz.

**Concomitant Phase**

**Adjuvant (maintenance) Phase**

Courtesy, Roger Stupp, Oct07
Frequency of *MGMT* Methylation in Glioblastoma

Range published for GBM 34% to 68% (gel based, mostly on frozen tissue)

<table>
<thead>
<tr>
<th>Tumor type</th>
<th># samples</th>
<th>MGMT-meth</th>
<th>%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBM</td>
<td>29</td>
<td>10</td>
<td>34</td>
<td>{Watanabe, 2005 #3670}</td>
</tr>
<tr>
<td>GBM</td>
<td>21</td>
<td>8</td>
<td>38</td>
<td>{Balana, 2003 #3672}</td>
</tr>
<tr>
<td>GBM</td>
<td>29</td>
<td>12</td>
<td>41</td>
<td>{Esteller, 2000 #1455}</td>
</tr>
<tr>
<td>GBM</td>
<td>12</td>
<td>5</td>
<td>42</td>
<td>{Yu, 2004 #2165}</td>
</tr>
<tr>
<td>GBM</td>
<td>74</td>
<td>33</td>
<td>45</td>
<td>{Kamiryo, 2004 #3677}</td>
</tr>
<tr>
<td>GBM</td>
<td>206</td>
<td>92</td>
<td>45</td>
<td>{Hegi, 2005 #2000}</td>
</tr>
<tr>
<td>GBM</td>
<td>44</td>
<td>30</td>
<td>68</td>
<td>{Blan, 2004 #3674}</td>
</tr>
<tr>
<td>GBM</td>
<td>38</td>
<td>26</td>
<td>68</td>
<td>{Hegi, 2004 #1721}</td>
</tr>
<tr>
<td>GBM</td>
<td>219</td>
<td>126</td>
<td>58</td>
<td>{Criniere, 2007 #6708}</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>672</strong></td>
<td><strong>342</strong></td>
<td><strong>51</strong></td>
<td></td>
</tr>
</tbody>
</table>
Comparison of qMSP and Classic Gel Based Nested MSP for determination of the *MGMT* status

<table>
<thead>
<tr>
<th>Quantitative MSP</th>
<th>Gel Based MSP</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OncoMethylome Sciences</strong></td>
<td>Lab of Tumor Biology and Genetics, Neurosurgery, CHUV</td>
<td>NCCR Molecular Oncology &amp; Swiss Institute of Bioinformatics</td>
</tr>
<tr>
<td>Ilse Vlassenbroeck</td>
<td>Annie-Claire Diserens Marie-France Hamou Monika E. Hegi</td>
<td>Eugenia Migliavacca Mauro Delorenzi</td>
</tr>
<tr>
<td>Stéphane Califice Josef Straub Ivano Di Stefano Fabrice Moreau Isabelle Renard, Bruno Flamion James DiGuiseppi Katja Bierau</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Tissues from Trials:** Lausanne, R. Stupp; Rotterdam, M. van den Bent Regensburg, P. Hau

Vlassenbroeck *et al* submitted
Experimental Workflow of the Assays

- **Evaluation of tissue** (H&E slide, tumor content, amount)
- **4 sections / sample for each center**

**GEL BASED ASSAY CHUV**
- Bisulfite treated DNA
- Nested MSP: 1st PCR (298bp)
- 2nd PCR (discriminating)
  - PCR for *meth MGMT* (81bp)
  - PCR s for *unmeth MGMT* (93bp)
- Results visualized on gel

**qMSP OncoMethylome Sciences**
- Bisulfite treated DNA
- quantitative MSP:
  - *meth MGMT* (136bp)
  - beta Actin (125bp)
- Ratio of mMGMT/Actin *1000

Comparison of results

Hegi 9-07
Non-disclosed Unpublished Data

Vlassenbroeck et al submitted
MGMT immunohistochemistry in GBM: Interobserver agreement in EORTC/NCIC trial 26981/22981

Methods: 2 anti-MGMT antibodies, **Dako MT3.1, Zymed MT23.2**

- Tissue micro array (TMA): 163 tissue samples from GBM trial 26981/22981
- 4 neuropathologists - 3 laboratories (RJ, GR, JAH, MP)
- Statistical analysis
New Molecular Targets

MGMT methylation Status

Frozen Tissue

RT/TMZ

Meth

Unmeth

months

Gene Expression Profiles

Array-CGH

18S

28S

Fluorescence

Time (seconds)

0 1 2 3 4 5 6 7 19 24 29 34 39 44 49 54 59 64 69

CHR 7
Non-disclosed Unpublished Data
Conclusions

• The *MGMT* methylation status predicts benefit from the alkylating agent TMZ

• Standardized *MGMT*-testing required
  – Quantitative MSP is reproducible, prospective testing ongoing
  – IHC is not useful for diagnostic MGMT-testing

• New trials will select patients based on *MGMT* status
The Team in the Lab

Anastasia Murat
Wanyu Louis Lambiv
Isabelle Desbaillets
Annie-Claire Diserens
Marie-France Hamou
Yan Lachat
Sophie Shnaper
Monika Hegi
Nicolas de Tribolet
Marc Levivier
Weizmann Institute of Science
Tal Shay
Eytan Domany

NCCR Molecular Oncology
ISREC
Eugenia Migliavacca
Mauro Delorenzi

NCCR Frontiers in Genetics
Genève
Patrick Descombes
Didier Chollet

UCSF
Anjan Misra
Burt Feuerstein

Oncology, CePO, CHUV
Roger Stupp
EORTC
Thierry Gorlia

Patients and their Families

EORTC
European Organisation for Research and Treatment of Cancer

Brain Tumor Group
Radiotherapy Group

National Cancer Institute of Canada

85 CENTERS
Michael Weller
Tübingen, D
Max Kros
Rotterdam, NL
Johannes Hainfellner
Vienna, A
Warren Mason,
Toronto, CA
Luigi Mariani
Berne, CH
Jacoline Bromberg
Utrecht, NL
Peter Hau
Regensburg, D
Gregory Cairncross
London, CA
René Mirimanoff
Lausanne, CH

The Jacqueline Seroussi Memorial Foundation for Cancer Research

SIAK
Personalizing cancer treatment

Fondation Nelia et Amadeo Barletta

NCCR Molecular Oncology