

Review

# Contribution of DNA repair mechanisms to determining chemotherapy response in high-grade glioma

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## Abstract

Despite the existence of a well described, succinct pathological grading system for gliomas, tumour behaviour between individual patients varies widely. In addition, predictors of response to treatment in glioblastoma multiforme are lacking. The majority of chemotherapeutic agents currently employed exert their effect on DNA. As our understanding of DNA repair mechanisms improves and predictive markers are elucidated, this may allow treating clinicians to individualise treatment based on molecular markers. This review examines important DNA repair mechanisms and their application to glioblastoma multiforme. By improving understanding of these mechanisms, and particularly the variations that occur between tumours and individuals, it may be possible to adapt treatment to maximise effectiveness and minimise toxicity.

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## 1. Introduction

Drug resistance involves a diverse group of genetic factors influencing various biochemical pathways. An intricate knowledge of the complex processes of DNA repair is important, as many treatments for glioma target tumour cells by attacking DNA. By understanding the inherent variability of DNA repair mechanisms in patients with glioma, robust molecular markers may be developed to define optimal treatment for individual patients.

In this review we briefly describe the impact and current treatment of glioblastoma multiforme (GBM), and then focus on recent developments in the molecular pathogenesis of these tumours, particularly in reference to DNA repair mechanisms. Understanding these changes in an individual patient may permit targeted therapy which maximises effectiveness while minimising toxicity.

## 2. Impact and current treatment of GBM

The social impact of high-grade glioma is disproportionate to its incidence. In recent data published by the Cancer Institute of New South Wales, malignant glioma accounted for only 1.5% of all new cases of cancer in males; however 2.9% of male cancer deaths were attributable to this disease (Cancer in New South Wales: Incidence and Mortality Report 2004, Cancer Institute NSW, [http://www.health.nsw.gov.au/cancer\\_inst](http://www.health.nsw.gov.au/cancer_inst)). Similarly in females, the figures were 1.3% and 2.6% respectively. What these statistics fail to demonstrate is that the average years of life lost for each patient with high-grade glioma is high (20.1 years), with one British study ranking it the highest of 17 malignancies.<sup>1</sup> Despite its relatively low incidence, high-grade glioma, and in particular GBM has a tremendous impact on the community.

The prognosis for patients with high-grade glioma remains poor. Surgery and radiotherapy have been the mainstay of treatment since the late 1970s. The role of

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radiotherapy is well established; more recent studies have been directed at optimisation of radiotherapy delivery and dosing regimens.<sup>2–4</sup> A number of chemotherapy agents and regimens have been trialled, with an extensive meta-analysis published in 2002 demonstrating a modest improvement in survival with chemotherapy.<sup>5</sup> Common chemotherapy agents currently employed in GBM are summarised in Table 1. A recent study conducted by the European Organization for Research on the Treatment of Cancer and the National Cancer Institute of Canada (EORTC/NCIC) demonstrated an improvement in median survival from 12.1 to 14.6 months with the addition of concurrent temozolomide to the previous standard of surgery and radiotherapy,<sup>6</sup> thereby establishing concurrent and adjuvant temozolomide as “standard of care” for GBM (Fig. 1).

Temozolomide has a primary mode of action of alkylation (or methylation) of the O<sup>6</sup> position of guanine residues in DNA, which subsequently leads to interstrand cross-linking, faulty DNA replication and cell death.<sup>7,8</sup> The EORTC/NCIC study utilised a regimen of concomitant temozolomide with radiotherapy (in an attempt to harness synergistic effects of these two treatments), followed by a 6-month course of temozolomide.<sup>6</sup> The findings of this study have led to the Pharmaceutical Benefits

Scheme (PBS) approval of temozolomide for use in newly diagnosed GBM in Australia.

Perhaps more interestingly, the EORTC/NCIC study demonstrated an increase in the proportion of patients surviving 2 years from 10.4% to 26.5% with temozolomide treatment.<sup>6</sup> This implies that in those patients who responded to temozolomide, there was some durability of response. It was suggested that this was related to the DNA repair enzyme O<sup>6</sup> methylguanine-DNA methyltransferase (MGMT). A study simultaneously published by Hegi et al. demonstrated that in patients where the response to temozolomide was pronounced, MGMT protein silencing by promoter methylation was more common.<sup>9</sup> This will be discussed more completely below.

These findings suggest that molecular markers such as MGMT offer enormous promise in determining the optimal treatment of patients with GBM. To identify additional markers, a complete and thorough understanding of the enzymes and mechanisms involved in DNA repair is required. DNA repair enzymes and mechanisms implicated in chemoresistance in glioma include MGMT, mismatch repair (MMR), base excision repair (BER) and the topoisomerase enzymes. These mechanisms, and their potential application to clinical decision-making, are discussed below.

Table 1  
Summary of commonly used chemotherapy agents in glioblastoma multiforme

Class of agent	Commonly employed agents	Mechanism of action
Alkylating agents	Temozolomide, BCNU (carmustine), CCNU (lomustine), carboplatin, procarbazine	Addition of methyl group to DNA residues
Antimicrotubule agents	Vincristine	Blocks formation of microtubules resulting in cell cycle arrest in metaphase
Topoisomerase I inhibitors	Irinotecan	Inhibit action of DNA repair enzyme topoisomerase I
Topoisomerase II inhibitors	Etoposide	Inhibit action of DNA repair enzyme topoisomerase II alpha
Anti-angiogenic agents	Thalidomide, bevacizumab	Inhibit formation of new tumour blood vessels

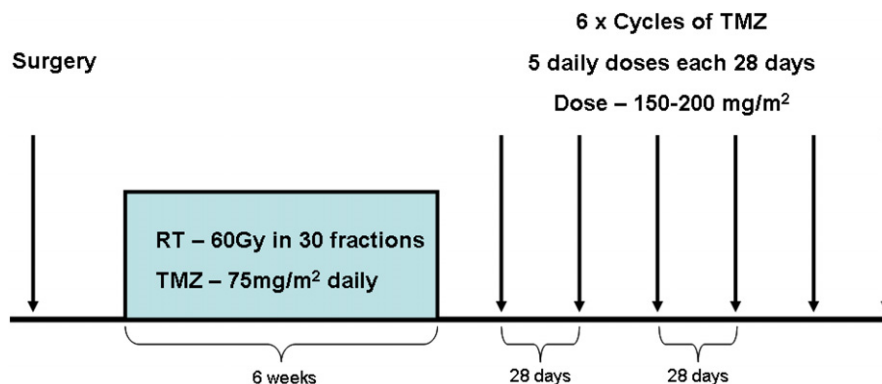


Fig. 1. Treatment of glioblastoma multiforme (GBM). Schematic representation of treatment of concurrent radiotherapy (RT) and temozolomide (TMZ) followed by adjuvant TMZ for patients with GBM. This regimen was trialled by the European Organization for Research on the Treatment of Cancer and the National Cancer Institute of Canada and has largely become the standard of care for GBM.

### 3. DNA repair mechanisms

#### 3.1. MGMT

Alkylating chemotherapeutic agents cause a variety of DNA lesions. The DNA adduct O<sup>6</sup>-methylguanine is one such lesion, and leads to interstrand cross-linking, faulty DNA replication and cell death.<sup>7,8</sup> The DNA repair enzyme MGMT is responsible for removal of an alkyl group from the O<sup>6</sup> position of guanine residues, and plays an instrumental role in DNA repair.<sup>10</sup> Essentially, the mode of action of MGMT is in direct opposition to that of temozolomide which causes methylation at the O<sup>6</sup> position of guanine (Fig. 2) and, not surprisingly, MGMT activity has been demonstrated to correlate inversely with the effectiveness of temozolomide chemotherapy in patients with high-grade glioma.<sup>11</sup> Epigenetic silencing of MGMT protein expression by promoter methylation has been frequently found in human neoplasia including glioma.<sup>12–23</sup> Methylation of the *MGMT* promoter, resulting in down-regulation of MGMT protein expression, has been shown in a number of studies to correlate with response to alkylating chemotherapy, including temozolomide.<sup>12,13,16–22,24</sup> As previously mentioned, Hegi et al. demonstrated that methylation of the *MGMT* promoter in patients with GBM resulted in markedly improved survival after temozolomide treatment when compared with patients in whom the *MGMT* promoter was unmethylated (21.7 vs. 12.7 months).<sup>9</sup> This study highlighted the potential importance of *MGMT* promoter methylation when considering which patients with GBM should be treated with temozolomide.

However, there are some issues that need to be resolved before *MGMT* promoter methylation can be implemented into common clinical practice. The exact incidence of *MGMT* promoter methylation varies according to the method of assessment and in different studies (Table 2).<sup>14,15,22</sup> Similarly the importance of tumour heterogeneity and changes in the methylation status of the *MGMT* promoter over time is unclear, with our group recently describing variation in the methylation status of the

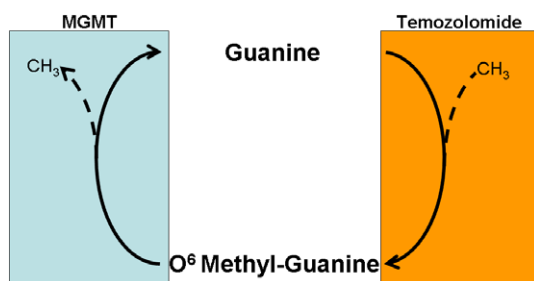


Fig. 2. Mechanisms of temozolomide and O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT). Actions of temozolomide (right panel) and MGMT (left panel). While temozolomide causes the addition of a methyl group at the O<sup>6</sup> position of guanine; MGMT removes this methyl group. CH<sub>3</sub> = methyl group.

Table 2

Overview of recently published studies examining O<sup>6</sup> methylguanine-DNA methyltransferase (MGMT) in glioma

Author	Method used	Incidence	
Esteller et al. (2000) <sup>12</sup>	MSP	19/47	40%
Balana et al. (2003) <sup>13</sup>	MSP	8/21	38%
Anda et al. (2003) <sup>14</sup>	IHC	10/18	55%
Yu et al. (2003) <sup>15</sup>	MSP	19/53	36%
Blanc et al. (2004) <sup>16</sup>	MSP	30/44	68%
Paz et al. (2004) <sup>17</sup>	MSP	28/92	45%
Hegi et al. (2004) <sup>18</sup>	MSP	26/38	68%
Kamiryo et al. (2004) <sup>19</sup>	MSP	33/74	45%
Watanabe et al. (2005) <sup>20</sup>	MSP	17/45	38%
Hegi et al. (2005) <sup>9</sup>	MSP	92/206	45%
Brell et al. (2005) <sup>21</sup>	MSP	51/93	55%
	IHC	47/93%	50%
Hassler et al (2006) <sup>22</sup>	MSP	3/12	25%
Maxwell et al (2006) <sup>23</sup>	MSP (Real time)	19/31%	61%
	IHC	10/32	31%
	Activity	12/32	38%

MSP = methylation-specific polymerase chain reaction; IHC = immunohistochemistry. Incidence refers to the incidence of MGMT promoter methylation, or in the case of immunohistochemical or activity studies, low MGMT expression or activity.

*MGMT* promoter both within the same tumour and also variation in methylation in the same tumour sampled on more than one occasion.<sup>25</sup>

The true potential of *MGMT* promoter methylation assessment lies in the stratification of patient treatment. Follow-up prospective clinical studies where different chemotherapy agents or other treatments are administered based upon *MGMT* methylation status will demonstrate the full utility of this marker. Watanabe et al. used the methylation status of the *MGMT* promoter to determine treatment in a small group of patients with high-grade astrocytomas.<sup>26</sup> In this study, patients whose tumours had methylation of the *MGMT* promoter were treated with procarbazine, nimustine and vincristine, while those who had an unmethylated promoter (and were thus likely to be resistant to alkylating agents) were treated with carboplatin and etoposide. This is, to our knowledge, the first example of prospective application of this technique to decision-making in high-grade glioma patients.

As a result of the recognition of the importance of MGMT activity as a chemoresistance mechanism, strategies have been developed to circumvent the resistance that MGMT confers. Combination therapy with multiple chemotherapeutic drugs known to deplete MGMT (specifically procarbazine and temozolomide) has been successfully assessed in a Phase I trial<sup>27</sup> but as yet has not been shown to confer a benefit in survival. O<sup>6</sup> benzylguanine (O<sup>6</sup>BG), a substrate for MGMT has also been used to decrease MGMT levels. However, systemic administration of O<sup>6</sup>BG has been associated with significant toxicity, thereby necessitating a reduction in chemotherapy dose.<sup>28,29</sup> A recent case report of local administration of O<sup>6</sup>BG, allowing the systemic effects to be avoided, shows some promise.<sup>30</sup>

Alteration of temozolomide dosing regimens from the usual 5 days every 28 days to more frequent, lower-dose treatment is also being evaluated. This so-called “metronomic” dosing has the theoretical advantage of depleting tumour MGMT protein, thereby increasing the effectiveness of temozolomide treatment. While Brock et al. demonstrated safety of a low dose of temozolomide for up to 49 consecutive days, the efficacy of this lower dose is unclear.<sup>31</sup> Depletion of peripheral mononuclear MGMT has been demonstrated with more prolonged dosing regimens and unfortunately this has been associated with profound lymphocytopenia and opportunistic infections.<sup>32–34</sup> More recent evidence suggests that daily dosing may in fact be associated with improved outcome.<sup>35</sup> The optimal dose and regimen for minimising toxicity but maximising MGMT depletion is yet to be determined, particularly the effect on outcome.

Further to the role of promoter methylation in the regulation of MGMT expression, more recent *in vitro* studies have searched for other factors which regulate MGMT. Two putative glucocorticoid response elements (GRE) have been noted in the promoter region of the MGMT gene.<sup>36</sup> GREs are regions in the promoter of a gene that in the presence of glucocorticoids lead to increased expression of the gene (Fig. 3). Treatment with dexamethasone could theoretically result in increased MGMT protein expression and subsequently increase resistance to alkylating agent chemotherapy. This has been demonstrated *in vitro*.<sup>37</sup>

Of additional interest is the transcription regulation protein p53 which is mutated in 30–50% of GBM. Inhibition of p53 activity has also been shown to enhance the sensitivity of some GBM cell lines to alkylating chemotherapeutic agents.<sup>38,39</sup> Interestingly, recent work in GBM cell lines has demonstrated some interaction between p53 and MGMT<sup>7,40</sup> with wild-type p53 expression resulting in increased expression of MGMT. This appears to be due to direct binding of p53 to the MGMT promoter and is irrespective of the methylation status of the promoter.<sup>7</sup> In the

future it may be necessary to consider MGMT and p53 status together when considering which patients are suitable for alkylator chemotherapy. Other regulators of MGMT remain unknown.

### 3.2. DNA MMR system

The DNA MMR system is an essential part of the process of correction of the rare but finite errors that occur during the process of DNA replication. Deficiencies in the MMR system can result in microsatellite instability (MSI), which is particularly important in hereditary non-polyposis colorectal cancer (HNPCC). MSI is relatively rare in glioma.<sup>41</sup>

However, the MMR system is important in resistance to alkylating agents. The O<sup>6</sup>-methylguanine adducts generated by these agents do not appear to be recognised directly by the MMR system; however, the subsequent mismatch of O<sup>6</sup>-methylguanine and thymine, which is normally cytotoxic to the cell, can be repaired by MMR.<sup>42</sup> It is this faulty repair and replication that is ultimately toxic to the cell (Fig. 4).

One of the most important genes involved in MMR is *human Mut L homologue 1 (hMLH1)*. Promoter methylation in the *hMLH1* gene has been shown to correlate with improved clinical response to nitrosoureas in malignant astrocytoma, with methylation occurring in 6/41 tumours examined.<sup>43</sup> Additionally, one study has demonstrated that high expression of hMSH2 protein correlated with resistance in GBM primary cultures; however, these primary cultures were intrinsically resistant to alkylating agents.<sup>44</sup> By contrast, mutations in the *hMSH6* gene have been shown to correlate with resistance to alkylating agents.<sup>45</sup> Friedman et al. did not show a significant difference in response to temozolomide with differing expression levels of MMR proteins hMLH1 and hMSH2.<sup>11</sup> Thus, the impact of MMR alterations on treatment response in GBM is conflicting and unclear.

While the impact of altered MMR on GBM resistance remains unclear, interplay between MMR and MGMT

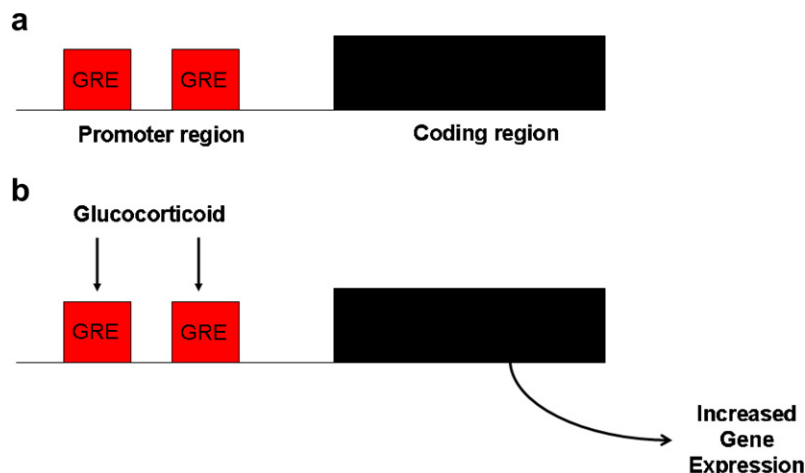


Fig. 3. Glucocorticoid response elements (GRE) are specific regions of the promoter of a gene (a). Glucocorticoids such as dexamethasone specifically bind to the GRE resulting in increased gene expression (b).

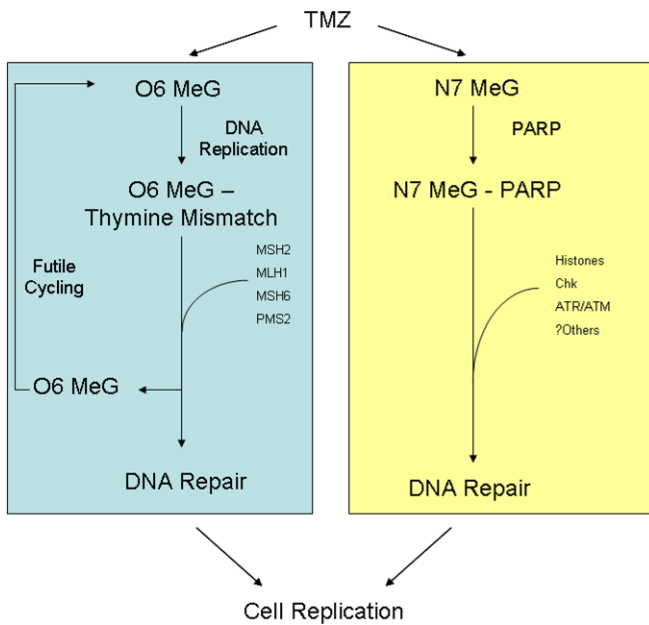


Fig. 4. Mismatch repair (MMR) and base excision repair (BER) in response to temozolomide (TMZ). Schematic overview of the interplay between the MMR system (left panel) and the BER pathway (right panel) in response to TMZ. When O<sup>6</sup>Methylguanine (O<sup>6</sup>MeG) lesions are generated as a result of TMZ treatment, an O<sup>6</sup>methylguanine – thymine mismatch arises leading to cell death. However, the repair enzymes MLH1, MSH2, MSH6 and PMS2 typically repair this mismatch with the subsequent futile cycling of this pathway resulting in cell death. The TMZ insult to DNA can also result in N<sup>7</sup> methylguanine (N<sup>7</sup> MeG) lesions. This leads to activation of the BER pathway, and the enzyme poly(ADP-ribose) polymerase (PARP) plays a critical role in the recruitment of co-factors to repair the base lesions. In the absence of the BER pathway, DNA strand breakage and faulty replication ensues, resulting in cytotoxicity.

has recently been identified. Fibroblast cell lines with low expression of MGMT were only sensitive to alkylating agents when hMLH1 was intact.<sup>46</sup> Even more interesting was an *in vitro* study that demonstrated that mutations in MMR were more important than MGMT status in conferring resistance to temozolomide, but had no effect on resistance to nitrosoureas.<sup>47</sup> This report suggests that in those patients whose tumours had high MGMT expression and low MLH1 expression, a nitrosourea may be a more appropriate choice for chemotherapy than temozolomide. Clinical studies whereby treatment is stratified based on MGMT and MLH1 may prove this to be the case.

### 3.3. Base excision repair

In addition to methylation at the O<sup>6</sup> position of guanine residues, temozolomide causes methylation at the N<sup>7</sup> position of guanine and the N<sup>3</sup> position of adenine residues. Despite the O<sup>6</sup> methylguanine adduct accounting for less than 10% of the methyl adducts formed by temozolomide, it is thought to be the predominant contributor to the cytotoxic activity of temozolomide.<sup>48</sup> The BER pathway is responsible for repairing the N<sup>7</sup> methylguanine and N<sup>3</sup> methyladenine lesions, and in particular the enzyme

poly(ADP [adenosine diphosphate])-ribose polymerase (PARP) plays a significant role in this (Fig. 4).<sup>49</sup> PARP has an important role in the nucleus in detecting DNA damage by catalysing the addition of ADP-ribose units to damaged DNA.<sup>50</sup> Increased activity of PARP has been noted after treatment of tumour cells with temozolomide and co-treatment of GBM xenografts with a PARP inhibitor and temozolomide has resulted in increased sensitivity when compared to temozolomide alone.<sup>51,52</sup> These findings are yet to be confirmed in clinical studies. It is quite possible that BER may also need to be considered when determining patient resistance to temozolomide treatment. Further clinical trials are eagerly awaited.

### 3.4. Topoisomerase enzymes

The topoisomerase enzymes play an important role in the conformational changes in DNA that are essential to DNA replication and transcription. DNA topoisomerase I (TopoI) functions as a swivel, allowing relaxation of DNA coils so that DNA replication and transcription can occur.<sup>53</sup> Topoisomerase II $\alpha$  (TopoII $\alpha$ ) creates transient breaks in double-stranded DNA, thereby allowing another double-strand to pass through this break.<sup>54,55</sup> This process allows intertwined DNA helices to separate.

#### 3.4.1. Topoisomerase I

The most commonly used topoisomerase-I inhibitor in glioma is CPT-11 or irinotecan. Xenograft models of high-grade glioma initially showed promising results for CPT-11 when used synergistically with alkylating agents.<sup>56</sup> However, subsequent studies examining CPT-11 as a single agent in recurrent high-grade glioma have been largely disappointing, with response rates of up to only 15%.<sup>57,58</sup> Prados et al. concluded recently that CPT-11 should not be used as single-agent therapy in recurrent high-grade glioma due to poor response,<sup>59</sup> although it is possible that this was due to inadequate dosing in those patients on enzyme-inducing anti-convulsants. Combination therapy with CPT-11 and other agents has also been implemented. A recent phase I trial has demonstrated the safety of CPT-11 and temozolomide in glioma, although the efficacy of this combination has not yet been determined.<sup>60</sup> CPT-11 has been used in combination with the vascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab in colorectal carcinoma and this combination has recently been applied to high-grade glioma.<sup>61</sup>

#### 3.4.2. Topoisomerase II

Since TopoII $\alpha$  is only expressed in the S and G2-M phases of the cell cycle, it has been utilised as a proliferative marker in both GBM and oligodendroglioma.<sup>62–67</sup> These studies have also demonstrated variable expression of TopoII $\alpha$  in GBM.

The chemotherapy drug etoposide exerts its effect via TopoII $\alpha$ . Etoposide inactivates TopoII $\alpha$  by increasing the

steady state concentration of its covalent DNA cleavage complexes. The effects of etoposide have been shown to be synergistic to the cisplatin analogue carboplatin, and this combination has been shown to be effective in recurrent GBM.<sup>55,68,69</sup> More recently the safety of combining temozolomide and etoposide has been evaluated in Phase I trials in GBM;<sup>70,71</sup> however, the efficacy of this combination still needs to be elucidated. It would be interesting to see if measurable TopoII $\alpha$  levels in patients could be used as a predictive marker for patient responsiveness to TopoII inhibitors.

#### 4. Patient treatment stratification

The difficulty with treating patients with GBM lies in the variability in both tumour behaviour and treatment response. As the predominant adjuvant treatments target DNA replication, the mechanisms of DNA repair inherent to these tumours are important in predicting response to current treatment. How these mechanisms vary between individuals with histologically identical tumours is integral to the tailoring of treatment.

Recent advances in molecular techniques have provided insight into how an individual may respond to treatment, in particular chemotherapy. The discovery of the loss of heterozygosity (LOH) of chromosome 1p and chromosome 19q and its importance in prognosis and chemosensitivity in oligodendroglioma has rapidly progressed into widespread clinical use.<sup>72</sup> While the best application of these techniques to clinical decision-making remains uncertain, the neuro-oncological community has embraced them.

Similarly, the use of *MGMT* promoter methylation as a predictive marker has also been met with enthusiasm. However, the most commonly applied technique to assess *MGMT* promoter methylation (methylation specific PCR) is technically difficult. In the largest study of *MGMT* promoter methylation to date (that performed by Hegi et al.), the analysis was only technically successful in 206 of 307 samples (67.1%) in which it was attempted.<sup>9</sup> A more reliable method of evaluation is needed before the application of this technique can become widespread. In addition, other factors need to be considered such as the interaction between p53 and *MGMT*. Despite this, the availability of such a marker that may prove both predictive and prognostic is exciting and encouraging.

As our understanding of DNA repair mechanisms and the role they play in chemoresistance improves, tailoring of therapy for individual patients may become feasible.

Undoubtedly, the multitude of small molecule inhibitors already in use and being developed will result in treatment for GBM becoming more individualised. Recent reviews in this journal have demonstrated a number of targets for both small molecule inhibitors and monoclonal antibodies.<sup>73–75</sup> The way in which these agents combine with those currently employed add further complexity to the treatment of this disease.

#### 4.1. Implications for oncology

As knowledge of molecular mechanisms has progressed, the treatment of cancer as a whole has changed. In many respects glioma is one of the solid tumours at the forefront of molecularly determined therapy. With the emergence of not only *MGMT* but also the recent recognition of potential molecular predictors of response to epidermal growth factor receptor (EGFR) inhibitors<sup>76</sup> to join the LOH of 1p/19q, a number of potential markers are becoming available to the neuro-oncological team.

Many of the mechanisms described above have an important role in tumours other than glioma. Indeed with further investigation, it is possible many of the principles of treatment stratification identified above could be applied to other cancers. The best such example of this is in hereditary non-polyposis colorectal carcinoma, where mutations in *MMR* genes result in microsatellite instability. Further, the response to chemotherapy is thought to depend on the presence or absence of microsatellite instability in these patients.<sup>77</sup>

*MGMT* methylation has also been implicated in a number of other cancers. These include hepatocellular carcinoma,<sup>78</sup> squamous cell carcinoma of the head and neck,<sup>79</sup> esophageal adenocarcinoma,<sup>80</sup> lymphoma,<sup>81</sup> colorectal carcinoma<sup>82</sup> and non-small cell lung carcinoma.<sup>8,83</sup> Studies correlating response to treatment with *MGMT* methylation in these cancers remain lacking.

The topoisomerases have also been recognised to be important in other tumours. In particular, overexpression of TopoII  $\alpha$  has been noted to correlate inversely with survival. Interestingly, TopoII $\alpha$  amplification or deletion has also been associated with amplification of the *HER-2* oncogene in breast cancer; such associations have not yet been recognised in glioma.

#### 5. Conclusion

Modest but significant advances have been made in the adjuvant treatment of high-grade glioma over the past 10 years. Simultaneously, the understanding of DNA repair mechanisms and how they relate to chemoresistance has improved enormously. What remains to be seen is how molecular methods are applied so that individualisation of treatment is possible, resulting in improved patient outcome with minimised morbidity.

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