Chemoprotection in glioblastoma therapy: reality or a dream?

Maciej M Mrugala^{*1,2}

Jennifer Adair²

Hans-Peter Kiem^{1,2}

“The requirement for a ‘chronic’ and safe administration of high-dose chemotherapy to fully eradicate tumor cells from the brain calls for unique measures...”

Glioblastoma (GBM) is one of the most deadly human cancers. Annually, over 10,000 individuals are diagnosed with this tumor in the USA alone [101]. The survival is dismal, and most patients succumb to the disease within 12–15 months from diagnosis. Brain tumors, unlike other cancers, do not respond well to conventional chemotherapeutic regimens and therapy for GBM is associated with a very high failure rate. Even the more recent introduction of novel antiangiogenic agents to therapy for GBM did not significantly improve prognosis for patients with malignant gliomas. There are several reasons that partially explain these disappointing therapeutic results. First, the blood–brain barrier limits the entry of many chemotherapeutics at conventional doses into the brain parenchyma and cerebro-spinal fluid [1]. Many agents that reach the brain parenchyma do not achieve high enough cytotoxic concentrations within the tumor. The most commonly used alkylating agent, temozolomide (TMZ), only achieves 20% of the serum area under the curve in the cerebrospinal fluid [1]. Microdialysis

experiments indicated that while TMZ can be found in the peritumoral brain parenchyma, concentrations of the drug might not be optimally therapeutic [2,3]. In addition to the structural blood–brain barrier, there are active efflux systems responsible for removing toxic compounds (including chemotherapy) from the brain tissue. Finally, and most importantly, approximately 50–60% of GBM tumors have an active enzymatic repair mechanism employing O-6-methylguanine-DNA methyltransferase (MGMT) to repair the cytotoxic DNA damage caused by alkylating agents such as carmustine (BCNU) and TMZ, efficiently protecting cancer cells from chemotherapy-mediated damage. High activity of the *MGMT* gene results in resistance to therapy and poor prognosis in patients with GBM [4]. Naturally occurring methylation of the *MGMT* gene promoter decreases expression of MGMT, rendering tumor cells susceptible to TMZ-induced cytotoxicity. As a result, patients with methylated (silenced) *MGMT* promoter in tumor cells display a better prognosis and response to therapy

“...one of the major barriers to using high doses of chemotherapy (and potentially maximizing efficacy) in cancer patients, including glioblastoma, has been its toxicity to vital organs...”

¹Department of Neurology, University of Washington Medical School, 1959 NE Pacific Street, Seattle, WA 98195, USA

²Fred Hutchinson Cancer Research Center, Seattle, WA, USA

*Author for correspondence: Tel.: +1 206 598 9487; mmrugala@u.washington.edu

with alkylating agents [4]. As such, the median survival in this group of patients is 21.7 months vs 12.7 months in the approximately 50% of patients with unmethylated (expressed) *MGMT* gene promoters. This latter patient population acutely needs novel approaches to therapy. Overcoming TMZ-resistance conferred by *MGMT* gene expression may be one of the solutions and could be achieved by using high doses of chemotherapy.

In general, high-dose chemotherapy regimens are rarely used in neuro-oncology. More commonly used agents that reach high concentrations in the CNS tissues include methotrexate, cytarabine and thiopeta. Unfortunately, these agents are not active against glioma. Nitrosoureas, such as BCNU, which are known to cross the blood–brain barrier and are toxic to glioma cells have been used; however, the high myelotoxic potential of these drugs significantly limits their utility, especially at high doses. In the 1980s, Hochberg *et al.* treated 11 patients with recurrent GBM with high-dose BCNU ranging from 600 to 1400 mg/m², followed by autologous stem-cell transplantation for hematopoietic rescue [5]. The median survival in this heavily pretreated cohort was 7 months and one patient died from the complications of therapy. Following this experience, high-dose chemotherapy approaches were not heavily pursued in therapy of malignant glioma, until recently.

In addition to using high-dose chemotherapy to overcome tumor resistance, direct inhibition of *MGMT* could be employed in combination with alkylating chemotherapy to restore therapeutic effects. The irreversible *MGMT* inhibitor O⁶-benzylguanine (O-6BG), which mimics the type of DNA damage caused by TMZ and repaired by *MGMT*, has been tested in combination with BCNU or TMZ in Phase I and II studies. Unfortunately, this combination (O-6BG plus BCNU or TMZ) produced dose-limiting hematologic toxicity, owing to a lack of *MGMT* expression in bone marrow stem and progenitor cells, preventing expansion of this approach into clinical practice [6–8]. Interestingly, local (intracavitary) administration of BCNU in a wafer form was not sufficient to completely overcome resistance of GBM, although systemic toxicity was not problematic with this approach [9]. It appears that glioma resistance to chemotherapy may require a more protracted treatment course, making conventional high-dose chemotherapy with autologous stem cell rescue an impractical approach. The requirement for a ‘chronic’ and safe administration of high-dose chemotherapy

to fully eradicate tumor cells from the brain calls for unique measures, such as chemoprotection of the bone marrow precursor cells and their progeny via induced expression of *MGMT* in this tissue.

As previously stated, one of the major barriers to using high doses of chemotherapy (and potentially maximizing efficacy) in cancer patients, including GBM, has been its toxicity to vital organs, primarily bone marrow. Patients receiving chemotherapy develop cytopenias leading to secondary complications such as infections or bleeding. Discontinuation of chemotherapy, delay in treatment or dose reductions are often necessary and may lead to decreased efficacy of the treatment. In the case of alkylating agents such as TMZ, bone marrow toxicity may be exacerbated by low expression of *MGMT*, which, as described above, can be upregulated in GBM cells, making the tumor chemoresistant. This is a challenging therapeutic dilemma where chemoresistant tumor cells and chemosensitive bone marrow cells coexist in one individual.

The ideal approach would employ therapy capable of killing GBM cells while simultaneously preventing bone marrow toxicity. A gene therapy strategy wherein creation of hematopoietic stem cells that carry a point-mutant *MGMT* gene (P140K), confers resistance to O-6BG but displays the same activity as wild-type *MGMT*, which could protect these cells and all their progeny from the toxic effects of chemotherapy after autologous transplantation (chemoprotection). Both *in vitro* and *in vivo* testing of O-6BG-resistant *MGMT* P140K gene-modified bone marrow stem and progenitor cells confirmed the feasibility of this approach [10–13]. Interestingly, therapy combining O-6BG with an alkylating agent also mediated selection of transplanted chemoprotected hematopoietic cells in preclinical models, increasing the population of gene-modified cells *in vivo* over time and allowing for long-term treatment with escalated doses of chemotherapy. Recently, this approach has been studied clinically in the unmethylated *MGMT* promoter GBM population. Our group showed that transplantation of hematopoietic stem cells genetically modified to express *MGMT* P140K can successfully protect bone marrow from the toxic effects of combination O-6BG and TMZ chemotherapy and potentially increase survival in this population [14]. Adair *et al.* reported that infusion of P140K gene-modified hematopoietic stem cells was well

“The ideal approach would employ therapy capable of killing glioblastoma cells while simultaneously preventing bone marrow toxicity.”

tolerated and resulted in higher levels of MGMT. P140K-modified blood cells after nonmyeloablative conditioning with BCNU (600 mg/m²) as a single agent. Patients who received transplantation of genetically modified hematopoietic stem cells were able to receive myelotoxic doses of TMZ (472 mg/m² or greater) when given in combination with O-6BG, the maximum tolerated dose defined in previously described Phase I and II studies, without permanent and irreversible toxicity. Up to nine monthly cycles of this regimen were provided and very encouraging survival results in this small group of patients were reported. The longest surviving patient is currently more than 36 months from the original diagnosis without signs of disease progression. This strategy thus produced very hopeful results in the cohort of patients with chemoresistant GBM and poor predicted response to standard therapy. While the efficacy results from this small study have to be interpreted cautiously, the feasibility of gene modification to achieve hematopoietic chemoprotection in clinical practice opens new research avenues and gives us a novel tool for cancer therapy. It is conceivable that using this method to treat less chemoresistant, yet still incurable cancers might result in very good outcomes. In GBM, treating patients with methylated MGMT promoter status using this approach might be particularly beneficial, as these tumors are more chemosensitive and more robust responses would be expected. While this novel approach to the treatment of cancer is extremely promising, gene therapy in GBM is rather distant from wide use in clinical practice. Larger, validating studies are needed to confirm safety and, in particular, efficacy of this therapy. The major concern with regard to the safety of gene modification has been insertional mutagenesis and the development of leukemia. However, the risk of this complication heavily depends on the transgene used and the disease background and is likely much reduced with newer vector systems available for gene transfer. In addition, even

the P140K-expressing γ retroviral vector used in the study mentioned above has been safe and has not resulted in any serious side effects. While technical aspects of described gene therapy are rather straightforward, the cost of such therapy remains high. Overall, however, it is comparable to the cost of nonmyeloablative stem cell transplantation used routinely in the therapy of hematologic malignancies. Moreover, it would be expected that gene therapy in GBM would eliminate the need for expensive sequential drug treatments currently used in clinical practice, and reduce the need for management of chemotherapy related side effects, thus potentially decreasing the overall cost of therapy.

Chemoprotection of hematopoietic stem cells in therapy of chemoresistant cancers such as GBM offers a novel and exciting clinical opportunity. Fine-tuning of this approach would likely lead to improved outcomes not only for GBM patients, but also in other cancers with high expression of MGMT. This strategy could also be beneficial for patients with recurrent GBM after initial treatment with TMZ fails, allowing for dose escalation of alkylating agents and hopefully leading to increased efficacy.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH nor its subsidiary Institutes and Centers.

Financial & competing interests disclosure

The authors are grateful for research funding from the NIH grant R01CA114218. H-P Kiem is a Markey Molecular Medicine Investigator and the recipient of the José Carreras/ E Donnell Thomas Endowed Chair for Cancer Research. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

- Ostermann S, Csajka C, Buclin T *et al.* Plasma and cerebrospinal fluid population pharmacokinetics of temozolomide in malignant glioma patients. *Clin. Cancer Res.* 10, 3728–3736 (2004).
- Blakeley J, Portnow J. Microdialysis for assessing intratumoral drug disposition in brain cancers: a tool for rational drug development. *Expert Opin. Drug Metab. Toxicol.* 6, 1477–1491 (2010).
- Portnow J, Badie B, Chen M, Liu A, Blanchard S, Synold TW. The neuropharmacokinetics of temozolomide in patients with resectable brain tumors: potential implications for the current approach to chemoradiation. *Clin. Cancer Res.* 15, 7092–7098 (2009).
- Hegi ME, Diserens AC, Gorlia T *et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. *N. Engl. J. Med.* 352, 997–1003 (2005).
- Hochberg FH, Parker LM, Takvorian T, Canellos GP, Zervas NT. High-dose BCNU with autologous bone marrow rescue for recurrent glioblastoma multiforme. *J. Neurosurg.* 54, 455–460 (1981).

“...it would be expected that gene therapy in glioblastoma would eliminate the need for expensive sequential drug treatments...”

- 6 Friedman HS, Pluda J, Quinn JA *et al.* Phase I trial of carmustine plus O6-benzylguanine for patients with recurrent or progressive malignant glioma. *J. Clin. Oncol.* 18, 3522–3528 (2000).
- 7 Quinn JA, Desjardins A, Weingart J *et al.* Phase I trial of temozolomide plus O6-benzylguanine for patients with recurrent or progressive malignant glioma. *J. Clin. Oncol.* 23, 7178–7187 (2005).
- 8 Quinn JA, Jiang SX, Reardon DA *et al.* Phase II trial of temozolomide plus O6-benzylguanine in adults with recurrent, temozolomide-resistant malignant glioma. *J. Clin. Oncol.* 27, 1262–1267 (2009).
- 9 Westphal M, Hilt DC, Bortey E *et al.* A Phase III trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro. Oncol.* 5, 79–88 (2003).
- 10 Beard BC, Sud R, Keyser KA *et al.* Long-term polyclonal and multilineage engraftment of methylguanine methyltransferase P140K gene-modified dog hematopoietic cells in primary and secondary recipients. *Blood* 113, 5094–5103 (2009).
- 11 Beard BC, Trobridge GD, Ironside C, McCune JS, Adair JE, Kiem HP. Efficient and stable MGMT-mediated selection of long-term repopulating stem cells in nonhuman primates. *J. Clin. Invest.* 120, 2345–2354 (2010).
- 12 Schambach A, Baum C. Vector design for expression of O6-methylguanine-DNA methyltransferase in hematopoietic cells. *DNA Repair (Amst.)* 6, 1187–1196 (2007).
- 13 Ragg S, Xu-Welliver M, Bailey J *et al.* Direct reversal of DNA damage by mutant methyltransferase protein protects mice against dose-intensified chemotherapy and leads to *in vivo* selection of hematopoietic stem cells. *Cancer Res.* 60, 5187–5195 (2000).
- 14 Adair JE, Beard BC, Trobridge GD *et al.* Extended survival of glioblastoma patients after chemoprotective HSC gene therapy. *Sci. Transl. Med.* 4(133), 133ra57 (2012).

■ Website

- 101 Central Brain Tumor Registry of the United States.
www.cbtrus.org/2012-NPCR-SEER/CBTRUS_Report_2004–2008_2013–2023–2012.pdf