

Recent clinical trials in diffuse intrinsic brainstem glioma

Review Article

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Abbreviations: complete response, (CR); diffuse intrinsic brainstem glioma, (DBSG); epidermal growth factor receptor, (EGFR); glioblastoma multiforme, (GBM); histone deacetylase, (HDAC); phenylacetylisoglutamate, (isoPG); neurofibromatosis 1, (*NF1*); *O*⁶-methylguanine-DNA-methyltransferase, (*MGMT*); phenylacetylglutamate, (PG); partial response, (PR); phenylacetate, (PN); phenylbutyrate, (PB); platelet-derived growth factor, (PDGF); progressive disease, (PD); stable disease, (SD); tissue inhibitor of metalloproteinase-3, (*TIMP-3*); transforming growth factor β , (TGFB)

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Summary

Brainstem gliomas constitute from 10 to 20% of all primary tumors of the central nervous system, and are diagnosed primarily in children. The most common variety is diffuse intrinsic glioma (DBSG), which occurs in approximately 85% of cases and creates a great challenge for neuro-oncologists. DBSG are inoperable and the only palliative treatment recommended is standard radiation therapy. Despite the treatment given, less than 10% of these patients will survive 2 years. Cytotoxic chemotherapy, including the newest agent temozolomide is not effective and the recently introduced targeted therapies with imatinib mesylate, gefitinib, erlotinib, and antiangiogenic therapy did not produce better results. The prognosis is especially dismal for recurrent DBSG with estimated survival of less than 6 months, regardless of the treatment given. Multitargeted therapy with antineoplastons A10 and AS2-1 (ANP), currently in clinical trials, has produced encouraging results in newly diagnosed DBSG. New strategies employing multitargeted approach may offer better results in the future. This review article summarizes the results of recent clinical trials in DBSG.

I. Introduction

Brainstem gliomas represent from 10 to 20% of all primary tumors of the central nervous system. They are diagnosed primarily in children and the median age of occurrence for all brainstem gliomas is 6 to 7 years (Fisher et al, 2000). The population of children suffering from these tumors in the U.S. is approximately 660 (CBTRUS, 2006). Less than 20% of brainstem tumors occur in adult patients. The brainstem controls extremely important functions of the body, including the heart rhythm and breathing. Due to the strategic location, it is easy to imagine that surgery and even biopsy of the brainstem may endanger a patient's life and create life-threatening complications. Stereotactic biopsy is associated with a limited risk in proficient hands, but a small specimen obtained may not be sufficient for a correct diagnosis; for instance, it may diagnose low-grade astrocytoma, when in fact the patient may suffer from both low-grade and high-grade tumors. Since the pathology diagnosis is difficult to obtain, dangerous and misleading, diagnosis based on magnetic resonance imaging (MRI) is the standard of care

(Freeman and Farmer, 1998). Although MRI and PET imaging is commonly used, it does not accurately replace histological diagnosis (Massager et al, 2000). Based on MRI, four different types of brainstem gliomas are identified: focal, dorsal exophytic, cervicomedullary and diffuse intrinsic (DBSG) (Freeman and Farmer, 1998). In addition, brainstem glioma associated with neurofibromatosis type I, is recognized by some authors as a separate entity because of the mild course (Guillamo et al, 2001). The most common variety is DBSG, which account for up to 85% of brainstem gliomas, and is most difficult to treat. For brainstem glioma associated with neurofibromatosis type I, no treatments are recommended unless there is rapid tumor growth and symptomatic worsening in the patient's condition (Pollack et al, 1996; Guillamo et al, 2001). Therapy for focal, dorsal exophytic and cervicomedullary tumors is similar to other types of primary brain tumors (Freeman and Farmer, 1998). For these reasons, this review will be limited to phase II/III studies in DBSG. Due to limited number of peer reviewed publications some data were collected from abstracts of

meetings. Diagnosing brain tumors is an unpleasant event for a clinician and a tragic one for the patient. Unfortunately, even more disappointing are the results of the current treatment for DBSG (Fisher and Buffler, 2005). Clearly new methods of treatment are needed and targeted therapy raises the most hopes for effective future treatment.

II. Histopathology, genomic and epigenomic changes

A. Histopathology and genomic changes

DBSG typically originate in the pons and behave in an aggressive manner. The determination of frequency of histopathologic types is not accurate, since only approximately 25% of these patients have a biopsy. Autopsy results confirm that the majority are high-grade gliomas, usually anaplastic astrocytomas (AA) (Freeman and Farmer, 1998). Glioblastoma multiforme (GBM) and low-grade, or grade 2 astrocytoma, occur in lesser frequency. In infants, DBSG shows more aggressive characteristics. In young adults, low-grade gliomas occur in 46% of cases according to multicenter study involving 48 adults in France (Guillamo et al, 2001). In older adults, high-grade glioma, including GBM, is more common and accounts for 31% of cases. For obvious reasons, the results of treatment are better in young adults with low-grade pathology. The cells of brainstem glioma spread throughout the brainstem and invade surrounding areas, but metastases to the distant areas of the central nervous system or other parts of the body are rare.

Genetic abnormalities in DBSG were studied only in a small number of cases, since such patients usually do not have a biopsy (Sung et al, 2000; Gilbertson et al, 2003; Broniscer and Gajjar, 2004). Approximately 50% of patients had *TP53* tumor suppressor gene (p53) mutations (Louis et al, 1993). The majority of patients with high-grade, but less than 20% of patients with low-grade DBSG have epidermal growth factor receptor (EGFR) protein detected (Gilbertson et al, 2003). In approximately 50% of all cases, there was allelic loss in the long arm of chromosome 10 where *PTEN* tumor suppressor gene is located (Louis et al, 1993; Cheng et al, 1999).

The studies of genetic changes of the tumors located outside the brainstem are much more extensive than DBSG. It can be expected that similar abnormalities occur in the brainstem as well as outside this area, but it is possible that DBSG have specific genetic characteristics that make them more resistant to treatment. The activity of p53 is neutralized by MDM2 protein which promotes its degradation (Vousden and Lu, 2002). The amplification of *MDM2* gene was found in up to 15% of high grade glioma (Reifenberger et al, 1994). MDM2 is inhibited by p14^{ARF} (p14), which is the product of *CDKN2A* gene. From 40 to 70% of GBM and 25 to 50% of AA, were found to have homozygous deletions of *CDKN2A* (Mantani A et al, 2003). *RAS* mutations, which are common in other malignancies, have not been found in human astrocytomas (Zhu and Parada, 2002; Newton, 2003, 2004). Promotion of neoplastic growth in astrocytoma in absence of *RAS* mutation occurs through p21^{RAS} protein which can be

activated through growth factor-receptor tyrosine kinase (GF-RTK) – *RAS* pathway and loss of neurofibromatosis 1 (*NF1*) gene (Guha et al, 1997). *AKT* plays an important part in pathogenesis of high grade glioma and is very active in GBM, but amplification of *AKT* has not yet been described in human astrocytomas (Ramaswamy et al, 1999). *AKT* protein is inactivated by *PTEN*, which is deleted in the majority of high grade gliomas (Ramaswamy et al, 1999; Newton, 2004). It is proposed that malignant gliomas develop through step-wise accumulation of genetic changes (Pollack et al, 1996; Sonoda et al, 2001). Inactivation of p53 and retinoblastoma protein (RB), activation of *RAS* and expression of human telomerase reverse transcriptase (hTERT) convert normal human astrocytes to cells resembling AA. In GBM in addition to such changes, there is usually activation of *AKT* pathway and loss of *PTEN*. A number of different targets are inactivated by *AKT* including *BAD* protein, which results in suppression of apoptosis (Sonoda et al, 2001). Another promising area of antitumor activity is *MYCC* oncogene pathway. Aberrant expression of *MYCC* is found in numerous malignancies and is associated with aggressive characteristics (Herms et al, 2000; Pelengaris et al, 2002; Burzynski et al, 2005). *MYCC* promotes G1-S phase progression and inhibits terminal differentiation (Bouchard et al, 1999; Amati et al, 2001). Transforming growth factor β (TGF β) is involved in a complex network of several hundred genes, which could be activated or suppressed (Siegel and Massague, 2003). TGF β 1 has increased activity in high grade tumors and is down-regulated by *PTEN* (Li and Sun, 1997).

B. Epigenomic changes

Epigenomic changes, which include DNA methylation, chromatin remodeling and histone modification are frequent in gliomas and may constitute the first event leading to formation of DBSG (Liau et al, 1992; Ordway and Curran, 2002; Robertson, 2002; Jaenisch and Bird, 2003; Esteller, 2005). DNA methylation is an extremely important chemical process involving regulation of gene expression. When a small "core" region of the promoter extending to the transcription start is methylated, the gene is silenced (Ushijima, 2005). In malignant tumors, there are usually both global hypomethylation and hypermethylation in promoter regions of tumor suppressor genes. Numerous research groups confirmed aberrant hypermethylation in the promoters regions and silencing of the genes including *p15^{INK4b}* (p15), *p16^{INK4a}* (p16), *p14*, *p21^{WAF1/Cip1}* (p21), *TP53*, *TP73*, and *O⁶-methylguanine-DNA-methyltransferase (MGMT)* (Herman et al, 1995; Zardo et al, 2002; Gonzalez-Gomez et al, 2003; Hong et al, 2003; Sanson et al, 2004; Hegi et al, 2005). Aberrant methylation of p16 promoter occurs in approximately 25% of gliomas (Costello et al, 1996). Promoter of the tissue inhibitor of metalloproteinase-3 (*TIMP-3*) is hypermethylated in approximately 70%-80% of low and high-grade gliomas (Bachman et al, 1999). Putative tumor suppressor gene *SLC5A8* is silenced through aberrant promoter methylation in most human gliomas, including low and high grade tumors (Hong et al, 2005). The aberrant methylation of the promoter DNA

mismatch repair gene *hMLH1* occurs in a significant fraction of malignant astrocytomas (Fukushima et al, 2005). Since promoter methylation occurs frequently in astrocytomas, it may constitute one of the early steps in the development of DBSG (Zardo et al, 2002; Hong et al, 2003). Silencing of *MGMT* plays an important part in sensitivity to temozolomide and patients with *hMLH1*-methylated malignant astrocytomas have a better chance to respond to nitrosourea (Fukushima et al, 2005). Global hypomethylation was found in every type of neoplastic cells studied, both benign and malignant (Feinberg and Tycko, 2004). It was responsible for increasing activity of oncogenes *HRAS* and *cyclinD2*, and other genes associated with proliferation, such as *14-3-3σ* (Feinberg and Tycko, 2004). Decreased global methylation in brain tumors is associated with a higher grade, genomic instability and multi-drug resistance (Ehrlich, 2002). There is a close association of global hypomethylation and the activity of SWI/SNF complex, which includes INI1 (SNF5) subunit. Mutation of *INI1* tumor suppressor gene is typical for teratoid/rhabdoid tumors (AT/RTs) and some GBM (Feinberg and Tycko, 2004). On the other hand, INI1 plays an important part in chromatin remodeling. SWI/SNF forms complexes with RB, and histone deacetylase (HDAC) in G1 phase of the cell cycle and depresses transcription of cyclins E and A (Trouche et al, 1997; Zhang et al, 2002). The function of INI1 may involve recruitment of SWI/SNF to promoters and repression of transcription of cyclins D, A and E (Zhang et al, 2002). On the other hand, SWI/SNF chromatin-remodeling factors increase expression of some genes through inhibition of promoters methylation (Banine et al, 2005). Chromatin remodeling is closely connected with histone modification (Jaenisch and Bird, 2003; Ringrose and Paro, 2004). The modification changes lead to formation of "histone codes", which help to preserve transcriptional memory known as cellular identity of heritable patterns of gene expression (Sims and Reinberg, 2004). Epigenetic mechanisms are attractive targets for effective treatments for DBSG (Liau et al, 1992). Among most promising are inhibitors of methylation complex for tumor suppressor genes (inhibitors of methyltransferases), inhibitors of HDAC, selective global hypermethylating and promoters hypomethylating agents, and drugs effecting chromatin remodeling and INI1. HDAC inhibitors, sodium phenylbutyrate (PB), sodium phenylacetate (PN) and trichostatin A have been proposed for the treatment of gliomas, and PB and PN underwent phase I clinical trials (Samid et al, 1994; Gore et al, 1997; Chang et al, 1999; Carducci et al, 2001; Gilbert et al, 2001; Baker et al, 2002; Kamitani et al, 2002; Phuphanich et al, 2005).

With the development of new targeted therapies, there is a revival of interest in stereotactic biopsy in DBSG. It is important to know the link between clinical behavior and molecular events. However, molecular analysis on these points has not yet reached the conclusion.

III. Recent phase II/III studies

A. Radiation therapy

Radiation therapy remains the main treatment for newly diagnosed DBSG in children older than 3 years of age (Mandell et al, 1999; Guillamo et al, 2001; Broniscer and Gajjar, 2004). In addition to conventional fractionated radiation therapy of 54 Gy in daily fractions of 1.8 Gy through parallel opposed portals, a number of hyperfractionation protocols have been tried, with or without radio sensitizing agents (Allen et al, 1999; Mandell et al, 1999). Randomized multicentric phase III study conducted by a Pediatric Oncology Group (POG) compared conventional and hyperfractionated radiotherapy (Mandell et al, 1999). Two groups of children, newly diagnosed with DBSG, 66 in arm 1 and 64 in arm 2 were randomized for either conventional to a total dose of 5400cGy (arm 1) or hyperfractionated to a total dose of 7020cGy (arm 2) radiotherapy with cisplatin as a sensitizer. The survival in both groups at 2 years was 7%. The authors did not report on 5 years survival, but the 3 patients (2%) survived from 3 to over 4 years. There was only one case of complete response (CR) in each arm, 18 cases of partial response (PR) in arm 1, and 15 cases of PR in arm 2. Stable Disease (SD) was documented in 25 patients in arm 1 and 23 in arm 2. Progressive disease (PD) was determined in 13 patients in arm 1 and 12 patients in arm 2 (Mandell et al, 1999). It was concluded that hyperfractionated radiation therapy did not improve the time to progression and overall survival compared to conventional radiotherapy. Additional studies proved worse survival in combined chemotherapy and radiotherapy trials (Freeman et al, 2000). Combined treatment with chemotherapy and radiation therapy, including high dose busulfan and thiotepa with autologous bone marrow rescue and high dose tamoxifen with radiation therapy did not provide better results (Bouffet et al, 2000; Broniscer et al, 2000; Freeman et al, 2000). A number of different chemotherapy agents, including carboplatin, etoposide, vincristine, topotecan and cyclophosphamide together with granulocyte colony-stimulating factor were tried as pre-radiation chemotherapy and followed with 72 Gy of radiation (Jennings et al, 2002). Unfortunately, none of these treatments improved response rate, event free survival, or overall survival compared to radiation therapy with or without chemotherapy. Stereotactic radiosurgery was used in the treatment of 18 patients diagnosed with pilocytic astrocytoma of the brainstem (Bernier-Chastagner et al, 2005). Among these patients, 12 had solid circumscribed tumors, 2 had cystic tumors and 4 DBSG. The authors reported that one out of 4 DBSG completely resolved and the 3 remaining patients developed tumor progression. Targeted radiotherapy, which is using a molecular vehicle to selectively deliver a radionuclide to malignant cell population, may increase the chances of response, but it was not yet tested in phase II trials for DBSG (Zalutsky, 2004). The current recommendation for newly diagnosed DBSG is conventional radiation therapy given in fractions of 1.8 Gy to the average total dose of 54 Gy (Schulz-Ertner et al, 2000). Unfortunately, such treatment has no application for recurrent DBSG.

B. Chemotherapy

Chemotherapy alone, or in combination with radiation therapy (neoadjuvant, concomitant or adjuvant), was used for newly diagnosed tumors without providing additional benefits (Freeman and Perilongo, 1999; Guillo et al, 2001; Reddy, 2001; Finlay and Zacharoulis, 2005; Donaldson et al, 2006). Numerous chemotherapeutic agents have been tried, including nitrosoureas, procarbazine, 5-fluorouracil, hydroxycarbamide, mechlorethamine, vincristine, eflornithine, cisplatin, carboplatin, cytarabine, etoposide, cyclophosphamide, ifosfamide, dactinomycin D and methotrexate without providing substantial benefit. The example of pre-radiation chemotherapy was described earlier (Jennings et al, 2002). Combination treatment with 5-fluorouracil and lomustine before radiation and hydroxyurea and misonidazole concomitant with radiation therapy did not show better results than standard radiation therapy (McLaughlin et al, 1998). Istituto Nazionale Tumori in Italy conducted three consecutive trials from 1983 to 2003, which included 50 children. In study 1, the patients received concomitant chemoradiotherapy with etoposide, cytarabine, ifosfamide and actinomycin D. The patients in study 2 were treated with high doses of thiotepa, cisplatin, etoposide, cyclophosphamide, methotrexate and vincristine, followed by radiation. In study 3, cisplatin and etoposide was used for 1 to 3 days for 10 monthly courses and radiation therapy was given after the second course. It was concluded that there was no additional benefit from chemotherapy (Massimino et al, 2000; Massimino et al, 2003). The older trials are summarized by excellent review articles (Hargrave et al, 2006; Freeman and Perilongo, 1999; Reddy, 2001; Finlay and Zacharoulis, 2005; Donaldson et al, 2006). Despite elaborate combinations, the results of chemotherapy in brain tumors in general did not change substantially since 1978 until the introduction of temozolomide, which is now the most promising chemotherapeutic agent for brain tumors (DeAngelis, 2005). The most recent results of phase III trials in adults with newly diagnosed GBM treated with radiation therapy alone or radiation therapy with temozolomide followed by adjuvant temozolomide indicated significantly longer overall survival and progression-free survival for patients treated with combined radiotherapy and chemotherapy (Stupp et al, 2005). Unfortunately, practically all of the benefits of temozolomide were found in a group of patients whose GBM contained a methylated *MGMT* promoter (Hegi et al, 2005), but in GBM, the majority of patients do not have hypermethylation of *MGMT* promoter (DeAngelis, 2005; Hegi et al, 2005). In newly diagnosed DBSG, the most recent combination with temozolomide included two cycles of intravenous irinotecan (10 doses of 20 mg/m²/d separated by two days of rest per cycle) over 6 weeks followed by standard radiation therapy and five days schedule of temozolomide (200 mg/m²/d) four weeks after radiation therapy and continued for a total of six cycles. Among 33 patients (median age 6.4 years), all died of disease progression and the median survival was 12 months. The estimated 1 year survival rate was 48% (Broniscer et al, 2005). An additional phase II study (ACNS0126) for high grade glioma and newly diagnosed

DBSG to determine the efficacy of temozolomide given during radiation therapy and as adjuvant treatment was closed due to poor responses. The prognosis for response to chemotherapy in patients with recurrent DBSG is worse than for newly diagnosed tumors with no efficacy reports (Freeman and Perilongo, 1999; Reddy, 2001). High dose chemotherapy with busulphan, thiotepa, etoposide and carmustine with autologous bone marrow rescue did not provide any benefit as reported by three different groups (Freeman and Perilongo, 1999). The European studies with temozolomide documented 1 case of partial response, surviving 17 months, corresponding to approximately 6% objective response (Lashford et al, 2002). The results of clinical trials of radiation therapy in combination with chemotherapy for newly diagnosed DBSG since 1999 are summarized in **Table 1** (for studies accruing at least 20 patients). Earlier clinical trials were reviewed by other authors (Freeman and Perilongo, 1999; Reddy, 2001; Finlay and Zacharoulis, 2005; Donaldson et al, 2006; Hargrave et al, 2006). The results of additional phase II study on combination of radiotherapy, vincristine and oral etoposide were recently presented, but revealed even lower overall survival at 2 years (3%) than radiotherapy alone (Korones et al, 2007). A small study of eight patients treated with osmotic blood-brain barrier disruption chemotherapy demonstrated 2 partial responses and MST of 16.5 months (Hall et al, 2006). Two additional reviews of the results of different treatment modalities were published recently (de Aquino Gorayeb et al, 2006; Wagner et al, 2006). One of these reviews did not document improved overall survival with combination of radiotherapy with chemotherapy and tamoxifen (de Aquino Gorayeb et al, 2006), but the other has shown a slightly better overall survival with irradiation and intensive chemotherapy (Wagner et al, 2006).

Unfortunately, it can be concluded that the reported studies of the application of chemotherapy in DBSG present only historical value. Even rare "promising" reports were not later confirmed by the studies conducted in different institutions. There is no doubt that a major paradigm shift is required in the treatment of malignant gliomas, and especially DBSG (Fisher and Buffler, 2005).

C. Antiangiogenic therapy

Continuous growth of brain tumors requires increased angiogenesis. Currently over 30 angiogenesis inhibitors are the subjects of clinical trials in the United States. Thalidomide is the first antiangiogenic drug approved by the FDA. In a group of 36 patients with GBM and AA treated with thalidomide as a single agent, only two obtained partial remissions (Fine et al, 2000). Combination treatment with thalidomide and temozolomide after radiation therapy gave slightly better results (Baumann et al, 2004). In recurrent GBM, combination of thalidomide and carboplatin produced 10% of partial responses (Glass, 1999). In the treatment of DBSG, thalidomide was used with carboplatin and radiation therapy in 14 patients. There were no complete and partial responses, but 2 patients obtained stable disease (Goldman et al, 2004).

Table 1. Results of radiation therapy in combination with chemotherapy for newly diagnosed, diffuse, intrinsic brain stem glioma.

Author	Study Type	Patients Total No.	Treatment		Efficacy							
			Radiation Therapy Gy	Additional Chemotherapy	OS 2 yrs %	OS 5 yrs %	MST M	CR %	PR %	SD %	PD %	
Mandell et al, 1999	Phase III											
Arm 1		66	54	Cisplatin	7.1	0	8.5	2	31	44	23	
Arm 2		64	HF70	Cisplatin	6.7	0	8	2	29	45	24	
Bouffet et al, 2000	Phase II	35	54	HDB Busulfan, thiotepa	5	0	10	NA	NA*	NA	NA	
Broniscer et al, 2000	Phase II	29	54	HD Tamoxifen	NA	NA	10	0	36	50	14	
Jenning et al, 2002	Phase II											
Arm A		32	HF72	Carboplatin, etoposide, vincristine, GCSF	10	0	NA	0	15	30	42	
Arm B		31	HF72	Cisplatin, etoposide, cyclophosphamide, vincristine, GCSF	4	0	NA	0	14	28	40	
Doz et al, 2002	Phase II	38	54	Carboplatin Etoposide, cytarabine, ifosfamide, cisplatin, dactinomycin D	5	0	11	0	0	37	63	
Massimino et al, 2003	Metaanalysis	23	UNK	Trophosphamide, etoposide	4	4	13	NA	NA	NA	NA	
Wolff et al, 2005	Phase II	20	54		NA	0	8	0	25	33	42	
Broniscer et al, 2005	Multiinstitutional	33	56	Temozolomide, irinotecan	0	0	12	NA	NA	NA	NA	
Bernier-Chastagner et al, 2005	Phase II	32	54	Topotecan	5	0	8.3	0	40	NA	NA	

The response rates were based on evaluable patients.

CR - complete response
 GCSF - granulocyte colony stimulating factor
 HD - high dose tamoxifen
 HDB - high dose chemotherapy and autologous bone marrow transplantation
 HF - hyperfractionated
 M - months
 MST - median survival time
 NA - not available
 OS - overall survival
 PD - progressive disease
 PR - partial response
 SD - stable disease
 UNK - unknown

* 1 patient had radiological improvement

On the other hand, thalidomide-associated thromboembolic events were described in patients with high grade gliomas (Cavaliere et al, 2004). Targeted therapy with bevacizumab in combination with irinotecan was used in the treatment of 21 patients with relapsed high

grade glioma (Stark-Vance, 2005). The preliminary results were encouraging with 5% CR and 38% PR. Recently published results of phase II trial of bevacizumab and irinotecan in recurrent malignant glioma (without DBSG) have shown better responses with 61% of PR and MST of

40 weeks in GBM (Vredenburg et al, 2007). At the time of this report, there is no proof of efficacy of thalidomide or any other antiangiogenesis agent in DBSG, but it is expected that this type of treatment may produce better results in the future. One possible mechanism suitable for brainstem glioma using antiangiogenic therapy is metronomic chemotherapy (Kieran, 2005). One case of brainstem glioma demonstrated radiological response with this metronomic treatment using temozolomide and oral etoposide (Korones et al, 2005).

D. Targeted therapy

After successful introduction of imatinib mesylate for treatment of chronic myeloid leukemia, this agent has been also tested in brain tumors clinical trials. The drug inhibits RTK, and platelet-derived growth factor (PDGF) receptors, which are frequently overexpressed in GBM and AA. The results of the studies indicate limited activity. In one of these studies, 29 GBM and 19 AA patients were treated with imatinib. There were no complete responses and only 1 PR in GBM group, and no CR and PR in AA group. The treatment was complicated with intratumoral hemorrhage in 5 patients (Wen et al, 2004). In the study for recurrent GBM in 51 patients, there were only 3 PR (van den Bent et al, 2004). The results of the recently completed phase I trial of imatinib in children with newly diagnosed brainstem and recurrent malignant gliomas are encouraging enough to recommend a phase II trial (Pollack et al, 2007). Gefitinib represents a new class of targeted therapy that inhibits RTK activity of the EGFR. Phase II study of gefitinib in recurrent GBM in 57 patients have shown no objective responses, even though only 21% of these patients had measurable disease at treatment initiation (Rich et al, 2004). In another study for newly diagnosed GBM in 98 patients, gefitinib did not show better median 1 year survival than standard treatment (Uhm, 2004). Erlotinib is a highly specific and reversible inhibitor of EGFR tyrosine kinase. In a study using erlotinib as a single agent in recurrent GBM in 24 patients, there were 5 short-lasting PR with progression free survival (PFS) of 5 months (Vogelbaum et al, 2004). Imatinib, gefitinib and erlotinib do not appear to have antitumor activity in GBM and AA as single agents, but may produce synergistic effect with chemotherapy. No reports are available at this time on the effect of these agents in DBSG. Imatinib should be used in caution due to the probability of intratumoral hemorrhage (Wen et al, 2004).

E. Multitargeted therapy

In brain tumors, genomic and epigenomic mechanisms create highly complex network of genes, proteins and small molecules. It is unlikely that the approaches affecting a single target will produce meaningful responses in DBSG. Neoplastic stem cells resemble normal stem cells. They are resistant to therapeutic agents and have efficient DNA repair system and a long life span. Without elimination of these cells, the long term control of malignant growth is highly problematic (Dean et al, 2005). Different types of neoplastic cells present in DBSG, from low-grade to high-

grade require broad spectrum therapeutic approach, such as: restoration of cell cycle control, induction of apoptosis and interference with cellular and nuclear transport, and cell metabolism. This translates to the introduction of the regimen, which affects *RAS*, *AKT*, *TP53*, *PTEN*, *INI1*, *p21*, *MYCC* and apoptosis pathways. Research data coming from different laboratories suggest that ANP regimen (antineoplastons A10 and AS2-1) may accomplish this task. Antineoplaston A10 injections (A10) consist of synthetic phenylacetylglutamate sodium (PG) and phenylacetylisoglutamate sodium (isoPG) in a ratio 4:1 and antineoplaston AS2-1 (AS2-1) consists of PG and phenylacetate sodium (PN) in a ratio of 1:4. Phase II studies with ANP which included patients with DBSG began in 1988 (Burzynski, 2004a). Only a small number of patients with DBSG were involved in most of these studies, which dealt with a broad spectrum of primary brain tumors (Burzynski, 2004a). In 1996, phase II study of ANP in patients with brainstem gliomas was opened and is nearing completion (Burzynski et al, 2003, 2004a, 2007). The studies are conducted at Burzynski Clinic and monitored by the FDA and the Institutional Review Board (IRB). The most recent report describes results in children with newly diagnosed DBSG (Burzynski et al, 2007). Among 20 evaluable patients, 5 were diagnosed with high-grade gliomas. The overall survival (OS) at 2 years was 40% and at 5 years was 30%. A CR occurred in 30%, PR in 10%, SD in 20% and PD in 40% (Burzynski et al, 2007). The study is closed for accrual and the final results will be evaluated before the end of 2007. **Figure 1** illustrates the response to treatment with ANP of DBSG in a 7-year-old girl (Case No. 5787). Phase III protocol is currently under the FDA's review. The results are summarized and compared to standard radiation and cisplatin and temozolomide studies in **Table 2**.

The proposed antineoplastic activity of ANP in DBSG consists of targeted therapy affecting the *AKT2* and the *TGF β 1* pathways (PG), *RAS*, *TP53*, and *p21* (PN) MYCC (PG and PN), MAD (PG), INI1 (PG), and apoptosis (PG and isoPG). PG is formed in patient's liver from PN and PB, but does not share with PN and PB an inhibitory affect on HDAC. The details of these mechanisms have been described previously (Castillo et al, 1991; Liau et al, 1992; Adam et al, 1995; Liu and Samid, 1995; Shack et al, 1995; Danesi et al, 1996; Gorospe et al, 1996; Prasanna et al, 1996; Adam et al, 1997; Engelhard et al, 1997; Harrison et al, 1998; Ng et al, 2000; Witzig et al, 2000; Li et al, 2001; Burzynski et al, 2003, 2004a,b, 2005; Waldbillig and Burzynski, 2003; Burzynski, 2004b, 2006a,b).

IV. Discussion

DBSGs remain some of the most tragic diagnoses in oncology. It is the general opinion of neuro-oncologists that the results of treatment for DBSG constitute the worst response in all primary brain tumors. DBSGs occur usually in children, in whom brain tumors in general are the second most frequent malignancy, and the most common form of solid tumors. Due to poor response, the number of clinical trials in DBSG is small, and there is not much interest in bringing new agents into the approval

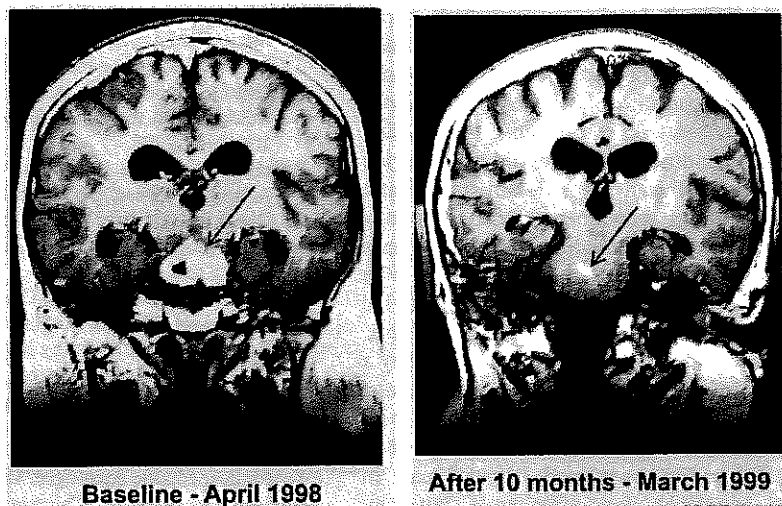


Figure 1. The response to treatment with ANP of DBSG in a 7-year-old girl (Case No. 5787).

Table 2. Results of treatment with antineoplastons A10 and AS2-1 (ANP) in patients with diffuse, intrinsic brain stem glioma compared to standard therapy.

Author	Study Type	Tumor Type	Number of patients	Radiation Therapy Gy	Treatment Additional Chemotherapy	Efficacy						
						ANP	OS 2 yrs %	OS 5 yrs %	MST M	CR % (No)	PR % (No)	SD % (No)
Mandel et al, 1999	Phase III	N	66 a	54	Cisplatin Temozolomide	7.1	0	8.5	2 (1)	31 (18)	44 (25)	23 (13)
Lashford et al, 2002	Phase II	R	21 b			NA	NA	6.2	0	5.6 (1)	15.7 (3)	77.8 (*4)
Broniscer et al, 2005	Multi institutional	N	33	55	Irinotecan	0	0	12	NA	NA	NA	NA
Burzynski et al, 2007	Phase II	N	20			ANP	40	30	16.4	30 (6)	10 (2)	20 (4)

N - newly diagnosed tumor
 R - recurrent tumor
 a - 57 evaluable patients
 b - 17 evaluable patients
 ANP - antineoplastons A10 and AS2-1
 OS - overall survival
 NA - not available
 MST - median survival time
 M - months
 CR - complete response
 PR - partial response
 SD - stable disease
 PD - progressive disease

* 1 patient had radiological improvement

process by the pharmaceutical companies. The results of only 6 clinical studies with chemotherapy have been published in peer-reviewed journals after the year 2000. Most of the results of phase II trials with targeted therapy and ANP have been presented at oncology meetings and published as abstracts. It was decided to include data from meeting abstracts in order to present the most up to date

results, but they should be treated with caution until they pass the scrutiny of peer review.

Children, older than 3 years, and young adults with newly diagnosed tumors, are usually temporarily helped with standard radiation therapy, but it is estimated that less than 10% of them will survive 2 years. Children, younger than 3 years, adults after the age of 40, and patients with high-grade glioma pathology have very grave prognosis,

and their median survival is similar to supratentorial GBM, or worse. Children diagnosed with DBSG and neurofibromatosis 1 have better prognosis, except those that show contrast-enhancement on MRI. Numerous regimens of standard chemotherapy, including temozolomide and high-dose treatment, did not provide better results and contributed to substantial toxicity; therefore, it is difficult to recommend them for newly diagnosed, as well as recurrent tumors. Antiangiogenesis therapy and newly introduced targeted therapy with imatinib mesylate, gefitinib, and erlotinib, did not provide better results and contributed to significant adverse events. Some of them, however; for instance, erlotinib, may play a more important part in combination with the other agents. The prognosis is especially grave for patients with recurrent DBSG, who typically do not survive longer than 6 months. Targeted radiotherapy and bevacizumab in combination with irinotecan may offer hope, but they would require further clinical trials. The patients with recurrent DBSG can be helped with treatments currently in phase II clinical trials. The results of phase II trials in DBSG with ANP compare favorably with responses and survival data in radiation therapy and chemotherapy trials. Currently conducted phase II studies are closed for accrual and nearing completion, and phase III studies are scheduled to open soon. Introduction of new multitargeted agents and acceleration of basic and clinical research in DBSG may offer better chances in the future.

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