A retrospective observational study on cases of anaplastic brain tumors treated with the Di Bella Method: A rationale and effectiveness

Giuseppe Di Bella¹, Vittoria Borghetto¹, Elena Costanzo¹
¹ Di Bella Foundation, Bologna, Italy

Correspondence to: Dr. Giuseppe Di Bella,
Di Bella Foundation, Via Marconi 51, Post code 40122, Bologna, Italy
TEL: +39 051 239662; +39 051230369, E-MAIL: posta@giuseppedibella

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Abstract
Despite all the new developments in cancer therapy, the life expectancy of patients with malignant anaplastic brain tumors and glioblastoma multiform (GBM) remains short. Since the establishment of the Di Bella Method (DBM) in cancer therapy, DBM was able to increase the survival rate and life quality, without overt toxicity, in comparison to what is described in the literature related to the analogous brain tumors, with the same immunohistochemical, histologic and clinical features. Therefore, we treated seven patients with malignant anaplastic brain tumors using the DBM protocol. DBM therapy consists of somatostatin and analogous (octreotide) all trans-retinoic acid (ATRA), β-Carotene, axerophthol dissolved in vitamin E, vitamin D, vitamin C, melatonin (MLT), proteoglycans-glycosaminoglycans, valproic acid, acetazolamide, diethyldithiocarbamate, hydroxyurea, and temozolomide. These molecules have either antiproliferative, antiangiogenic, cytostatic, antioxidant, antimetastatic (differentiative), and immunomodulating features. Moreover, the inclusion of ATRA, MLT, and glucosamine with sodium valproate, diethyldithiocarbamate and acetazolamide has reinforced antitumor properties of the therapy by extending them to cancer stem cells. Furthermore, the non-cytolytic and non-cytotoxic metronomic dosage of hydroxyurea and temozolomide had increased the DBM therapy outcome by strengthening anti-tumor capability. The results of such treatment revealed that all seven patients were still alive after 5 to 8 years of starting DBM. In conclusion, the multi-strategic objectives of DBM are inhibiting the proliferative-invasiveness and neoplastic angiogenesis, silencing the survival system of cancer stem cells, enhancing the immunomodulatory and antioxidant activities, improving vitality and efficiency of normal cells, and depressing the efficiency and vitality of neoplastic ones.

Abbreviations:
AAAZ - Acetazolamide
ATRA - All Trans Retinoic Acid
ALDH - Aldehyde Dehydrogenase
Ca 9 - Isoenzyme of CAH
CAH - Carbonic Anhydrase
CCK - Cholecystokininn
C.M. - GH-induced chemotaxis of Monocytes
CSC - Cancer Stem Cells
DBM - Di Bella Method
EGF - Epidermal Growth Factor
EGFR - Epidermal Growth Factor Receptor
FGF - Fibroblastic Growth Factor
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GBM  - Globlastoma Multiforme
gCSC - Globlastoma Cancer Stem Cells
GF  - Growth Factor
GH  - Growth Hormone
GHR - Growth Hormone Receptor
HDAT - Histone deacetylase
HGF - Hepatocyte Growth Factor
HIF-1α - Hypoxia-Induced Oncogenic Factor
IGF1-2 - Insulin-like Growth Factor 1-2
IGFR - Insulin-like Growth Factor Receptor
IL8 - Interleukin 8
MRI - Magnetic Resonance Imaging
MLT - Melatonin
NGF - Nerve Growth Factor
NHL - Non-Hodgkin’s Lymphoma
NOse - Endothelial nitric oxide synthase
PDGF - Platelet-Derived Growth Factor
PET - Positron Emission Tomography
PG2 - Prostaglandin 2
PRL - Prolactin
PRLR - Prolactin Receptor
RMN - Magnetic Resonance Imaging
SSN - National Health Service
SST - Somatostatin
SSR - Somatostatin Receptor
TGF - Transforming Growth Factor
TRK - Tyrosine-kinase
VEGF - Vascular Endothelial Growth Factor
VIP - Vasoactive Intestinal Peptide
VPA - Valproic Acid

INTRODUCTION

Malignant anaplastic brain tumors and glioblastoma multiforme (GBM) remain an unresolved clinical problem. Furthermore, GBM is the most frequent and aggressive type of cancer affecting different glial cells of an adult brain. Although there are advancements in the treatment of cancer, malignant brain tumors and GBM treatments fall short. For instance, Stupp's regimen consists of the main course for treating anaplastic astrocytoma and GBM patients. The regimen consists of surgery followed by radiotherapy. However, the overall median survival remains 15-18 months after diagnosis and has not significantly improved in the last decade (Delgado-López & Corrales-García 2016; Lakomy et al. 2020). In addition, total resection (above 98% of tumor volume) increases survival compared to subtotal or partial resection. On the contrary, “extended” subtotal resection does not confer any advantage compared with partial resection or biopsy (Laws et al. 2003). Generally, the one-year survival is 57%, decreasing to 16% at two years, and to 7% at three years (Filippini et al. 2008). However, GBM patient who lives more than three years is described as “long survivor”, and this condition is often limited to subtotal resection surgery.

Since cancer cells development is a multistep process involving multiple abnormal signaling and genetic pathways, treatment should be performed similarly. Also, cancer stem cells (CSC) should be targeted to halt the re-progression of cancer. Thus, the Di Bella Method (DBM) was established (Di Bella et al. 1979a; Di Bella et al. 1979b). The DBM consists of administering several specific molecules where each molecule is chosen based on its mechanism of action against tumor cells, CSC, proliferation and apoptosis, oncogenes, angiogenesis, molecular analysis, and genetic mutation. Besides, some molecules were chosen for their preservative mechanisms on healthy cells, including cell membrane integrity, DNA preservation, and mitochondrial function (Di Bella 2010).

For several years, we have been using the DBM for treating several types of cancer including breast, head and neck, and several others with success (Di Bella et al. 1979a; Di Bella et al. 1979b; Di Bella 1997; Di Bella & Di Bella 1998; Di Bella 2005; Di Bella & Gualano 2006; Di Bella 2010; Di Bella et al. 2013a; Di Bella et al. 2013b; Di Bella & Di Bella 2015; Di Bella et al. 2017; Di Bella et al. 2018; Di Bella 2019). In brain cancer, the percentage of CSC is associated with chemo and radio-therapy resistance, and thus rapid re-progression of the disease occurs. Since DBM protocol targets many steps of cancer development as well as CSC, we treated seven patients with malignant anaplastic brain tumors and GBM. In addition, we mentioned how DBM components halt tumor growth and enhance patients’ survival with a brain tumor.

DBM TREATMENT RATIONAL FOR MALIGNANT BRAIN TUMORS

The DBM therapy for brain tumors consisted of administering multiple drugs, vitamins and molecules as outlined in Table 1. Each of the drugs or vitamins or supplements used was based on our previous clinical results on different types of cancer (Di Bella et al. 1979a; Di Bella et al. 1979b; Di Bella 1997; Di Bella & Di Bella 1998; Di Bella 2005; Di Bella & Gualano 2006; Di Bella 2010; Di Bella et al. 2013a; Di Bella et al. 2013b; Di Bella & Di Bella 2015; Di Bella et al. 2017; Di Bella et al. 2018; Di Bella 2019).

Somatostatin and somatostatin analogues

In the DBM method, several molecules were used to halt cancer proliferation and growth. These molecules were chosen based on scientific research. For instance, cell proliferation, physiological or pathological, and protein synthesis is closely dependent on the interaction between prolactin (PRL) and growth hormone (GH). GH is the primary inducer of growth (De Souza et al. 1974; Lincoln et al. 1998; Friend 2000; Barnett 2003; Anthony & Freda 2009). GH is also the principal mediator of postnatal cell growth and differentiation in somatic cells following binding to its receptor, GHR (Le Roith et al. 2001; Zhu et al. 2001). This binding activates pivotal pathways of cell growth and survival, such as the JAK-2/STAT signaling pathway, p44/42 family of mitogen-activated protein kinases (MAPKs), and phosphoinositide 3-kinases (PI3Ks) family (Le Roith et al. 2001; Zhu et al. 2001). On the other hand, GHRs...
are widely distributed with varying concentrations in different cell types, normal as well as neoplastic ones. The fact remains - the more GHRs are expressed in tumor cells, the more tumor cells possess invasive and metastatic capabilities (Lincoln et al. 1994).

Besides, there are several GH-dependent mitogenic molecules, such as EGF, FGF, IGF1, VEGF, PDGF (Hagemeister & Sheridan 2008; Murray et al. 2004; Sall et al. 2004; Szepesházi et al. 1999; Taslipinar et al. 2009), and gastrointestinal (GI) specific growth factors, such as VIP, CCK, G (Kath & Höffken 2000) that are involved in many types of cancer. Furthermore, prolactin receptor (PRLR) and GH receptor (GHR) are co-expressed on cellular membranes, and through heterodimerization they can physically and functionally interact, amplifying proliferative pathways (Kelly et al. 1993). It has been found that GH and PRL have a key role in the development and in the progression of human tumors, especially in brain (De Souza et al. 1974; Ben-Jonathan et al. 2002; Batra et al. 1997; Cameron et al. 1979).

Tumor induction and progression are positively related to GHR-PRLR expression, as demonstrated by immunohistochemistry, Western blot, in situ hybridization and qPCR analyses. It's well documented that in tumor tissues GHR levels are higher than in physiological or peritumoral ones, confirming the mitogenic key role (Di Bella et al. 2018; Friend 2000; Gruszka et al. 2001; Lincoln et al. 1998; Zeitler & Siriwardana 2000). Although the exact timing of etiopathogenesis is not completely understood, it's conceivable that autocrine and/or paracrine signaling could be accountable for local production of GH, GHR, PRL, PRLR, and IGF1 in many tumors, including central nervous system ones.

IGF-1 is strongly dependent on GH (Daughaday & Trivedi 1987). Moreover, it's well known that GH administration causes an increase of IGF-1 in human tumor cells. A large amount of GHR could be observed in cell lines during exponential cell growth and GH/IGF-1 axes is the principal mediator of somatic growth and has a crucial role in oncogenesis (Laban et al. 2003),

<table>
<thead>
<tr>
<th>Tab. 1. DBM therapy for GBM</th>
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<tbody>
<tr>
<td><strong>DRUG</strong></td>
</tr>
<tr>
<td>Somatostatin</td>
</tr>
<tr>
<td>Octreotide LAR</td>
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<tr>
<td>Retinoid solution</td>
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<tr>
<td>Vitamin C</td>
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<tr>
<td>Vitamin D3</td>
</tr>
<tr>
<td>Synacthen*</td>
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<tr>
<td>Parlodel*</td>
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<td>Dostinex*</td>
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<tr>
<td>Chondroitin sulfate</td>
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<tr>
<td>Glucosamine</td>
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<tr>
<td>Deursil*</td>
</tr>
<tr>
<td>Melatonin</td>
</tr>
<tr>
<td>Temodal*</td>
</tr>
<tr>
<td>Oncocarbide*</td>
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<tr>
<td>Depakine Chrono*</td>
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<tr>
<td>Diamox*</td>
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<tr>
<td>Disulfiram</td>
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inhibiting apoptosis (Kusano et al. 2014; Perry et al. 2006), and stimulating the production EGF, FGF and VEGF-A (Cattaneo et al. 1999; Brunet-Dunand et al. 2009; Vacas et al. 2016).

Furthermore, GH promotes migration and invasiveness of tumor cells via the endothelial to mesenchymal transition (EMT) in tumor cells and down-regulating plakoglobin, the cytoplasmic relocation of E-cadherin and the activation of metalloproteinases 2 and 9 (MMP2 and MMP9) (Mukhina et al. 2004; Sommers et al. 1994; Thiery 2002). Also, GH transduction leads to a significant induction of several angiogenic genes, such as the endothelial nitric oxide synthase (eNOS), the vascular endothelial growth factor (VEGF), the basic fibroblast growth factor (bFGF). Additionally, using immunohistochemistry analyses, GH displays an increase in capillary density and cellular proliferation (Kusano et al. 2007).

According to some studies, mRNA of GHR was found in healthy brain cells and glioblastoma (Castro et al. 2000). Overexpression of GH and GHRH in cancer tissues such as glioblastoma, breast, lungs and others is well documented (Lincoln et al. 1998). Several studies confirmed the antitumor efficacy of somatostatin analogues in glioblastoma, mainly by inhibiting GH and GHRH release (Jaekle et al. 2003; Kovács et al. 2010).


Furthermore, the antiproliferative effect of somatostatin and somatostatin analogues takes place by inhibiting IGH pathways (Kiaris et al. 2005). Regression and long survival with somatostatin in a primary gliosarcoma, a type of GBM but rare and has poor prognosis, confirmed the efficacy of somatostatin in this type of pathology (Anthony & Freda 2009; Barnett 2003; De Souza et al. 1974; Friend 2000; Lincoln et al. 1998; Trignani et al. 2013).

In conclusion, the PRL/GH/IGF axis has a prominent role in malignant growth, providing a rationale for the use of anti-dopamine D2 receptor agonists in combination with biological GH antagonists, such as somatostatin and its analogues. Inhibiting such axis downregulates GH-related mitogenic growth factor, including IGF1 and 2 (Arena et al. 2007; Buckley & Buckley 2000), EGF (Barrie et al. 1993; Witt et al. 2008), FGF (Bonneteer et al. 1990), VEGF (Albini et al. 1999; Ashino et al. 2003; Cascini et al. 2001) PDGF (Cattaneo et al. 1999) and their relative pathways, resulting in anti-proliferative and pro-apoptotic signals for recipient cells (Watt et al. 2008). This emerging way of viewing is well documented in basic research, but it’s not yet translated in clinical applications.

Disulfiram (Aldehyde dehydrogenase inhibitor)

We have integrated DBM with Disulfiram, an aldehyde dehydrogenase (ALDH) inhibitor, known as a marker of glioblastoma CSCs (Toledo-Guzmán et al. 2019; Moreb 2008), that is involved in the renewal, differentiation and auto-protection (Marcato P et al. 2011; Marcato P et al. 2011). Glioblastoma CSCs (gCSC) represent the chemical-resistant population responsible for recurrence, with a relevant percentage in glioblastoma cellular population. The temozolomide (TMZ), an alkylating agent, is a first line chemotherapy drug, able to ameliorate patient survival, and the resistance to it leads to treatment failure (Schäfer A et al. 2012). It has been highlighted that “long noncoding RNAs” (lncRNAs)” are related to a significant positive regulation of TP73-AS1 in gCSC, related to overexpression of ALDH1A1, one of the predominant ALDH isozymes, protein (Nikhil et al. 2019), hallmark of ill-fated prognosis in GBM and in other cancer disease (Mazor et al. 2019). ALDH1A1 was found regularly in gCSC and it is associated with chemoresistance to TMZ (Moreb 2008). Biomolecular mechanisms involving ALDH1A1 were highlighted. In gCSC, overexpression of ALDH1A1 increases their aggressiveness and resistance, making them a target for therapeutic strategies (Safa et al. 2016; Xu et al. 2015).

gCSCs characterized by mesenchymal phenotype exhibit high intracellular of ALDH1 Family Member A3 (ALDH1A3) and are considered more aggressive and more resistant to therapy (Chen et al. 2019). In a lot of cancer types, such as breast and ovarian carcinomas, neuroblastomas, retinoblastomas, etc., the presence of CSC was demonstrated and related to overexpression of their specific marker ALDH1, leading to a poor prognosis, enhanced aggressiveness and chemoresistance (Flahaut et al. 2016; Kim et al. 2018; Marcato et al. 2011; Marcato et al. 2011; Seigel et al. 2015). In this context, the introduction in DBM of disulfiram, a negative regulator of ALDH is a rational choice. Moreover, Disulfiram is well tolerated without any toxicity at the dosage of 200 mg per day.

Valproic Acid

VPA, an anti-convulsion medication, has been shown to have antineoplastic activities by inhibiting histone deacetylase and chromatin condensation (Krauze et al. 2018), and allowing access to all transcription,
differentiation and cytostatic factors (Ruda et al. 2016). Moreover, VPA activates tumor suppressor genes (Garcia et al. 2018), and inhibits glioma proliferation in vitro and in vivo, by increasing apoptosis and inducing cell cycle arrest (Pan et al. 2017). Therefore, VPA was considered a valid therapeutic option for GBM treatment (Kerkhof et al. 2016) and potentiates the antitumor response with a lot of mechanisms, including differentiation in different types of tumor cells (Rudà et al. 2017). VPA modifies the expression of genes involved in differentiation, DNA repair and apoptosis. In particular, VPA induces p21 expression, blocks cell cycle in G2/S phase, and activates at the same time an apoptotic cascade, through downregulation of antiapoptotic protein Bcl-2 / Bcl-XL. Indeed, as reported in recent studies, a reduction of the mitotic index emerged after VPA treatment, confirming the induction of the G1 block. Commonly, cell arrested in G1 phase move toward differentiation and subsequent apoptosis (Riva et al. 2014). Clinically, VPA administration with temozolomide led to an increase in survival of GBM patients (Kerkhof et al. 2013).

VPA has been shown to counteract the proliferation and mobility of gCSCs (Gefroh-Grimes & Gidal 2016) and potentiates the antitumor response with a lot of mechanisms, including differentiation in different types of tumor cells (Rudà et al. 2016). In gCSCs, VPA induces a dose-dependent reduction of metabolic activity. The efficacy of this drug is revealed, evaluating the classification of gCSCs lines, from the more sensible to less responsive: G166, G179 and G144, GBM2, GBM7, GliNS2 (Riva et al. 2014).

Carboanhydrase inhibitors

The carbonic anhydrases (CAH), zinc metalloproteins, are enzymes that can have a clinical relevance in cancer therapy, because of their isoform specific for cell surface. The Ca9, an isoform of CAH, is almost exclusively associated to cancers, and it is involved in tumorigenesis. Ca9 is overexpressed in many types of cancer, and it is infrequently present in healthy tissues. Ca9 expression is induced by hypoxia and acidification, both of which present in neoplastic areas of solid tumors (Winum et al. 2008; Said et al. 2010; Said et al. 2013; Supuran & Winum 2015). Furthermore, overexpression of Ca9 represents a significant indicator of disease prognosis, associated with increased aggressiveness, malignant progression, metastasis and poor response to treatment documented in various tumors (McDonald et al. 2019). Therefore, the inhibition of Ca9, mediated by acetazolamide (AAZ), counteracts its carcinogenic role (Pastorekova et al. 2008).

Melatonin

Melatonin (MLT) is a natural hormone produced from the pineal gland and is associated with the control of sleep-awake cycle (Auld et al. 2017). Furthermore, MLT has antioxidant, anti-aging and immunomodulatory properties. MLT has a relevant role in the hematopoiesis, mainly thrombogenesis, leukocytes regulation, and synthesis of hemoglobin. Besides, MLT has a prominent role in perfusion and in gaseous haemato-tissue exchanges, preventing tissue ischemia, acidosis, and hypoxia in neoplastic environment, with a consequent over-expression of oncogenic genes, such as HIF-1α. The relevant and non-toxic apoptotic, oncostatic, anti-angiogenic, antiproliferative properties of this indole on all neoplastic pathologies are documented (Heldin & Westermark 1991; Cos et al. 1996; Lissoni et al. 1996; Bartsch & Bartsch 1997; Blask et al. 1997; Pawlikowski et al. 1999; Lissoni et al. 2000; Czeczuga-Semieniuk et al. 2002; Reiter & Korkmaz 2008; Sánchez-Barceló et al. 2005; Skwarlo-Sonta 2002; Trubiani et al. 2005; Vijayalaxmi et al. 2004; Watanabe et al. 2008; Fischer et al. 2008; Bejarano et al. 2009; Matt et al. 2009; Ferreira Cda et al. 2010; Kim et al. 2013; Moradkhani et al. 2020). Clinically, MLT showed potential in treating solid tumors (Di Bella 2005; Di Bella & Gualano 2006; Di Bella et al. 2013a; Di Bella et al. 2013b; Nooshinifar et al. 2017; Talib 2018; Di Bella 2019; Gil-Martín et al. 2019).

Retinoid solution

Retinoids are chemical compounds related to vitamin A. Early studies had shown that retinoids regulate cell proliferation and differentiation. Then, several studies demonstrated that retinoids play a crucial role both in prevention and therapy of cancer, limiting consequences induced by cancer and usual anticancer therapies (Abe et al. 2003; Adachi et al. 2001; Di Masi et al. 2015; Anthony & Freda 2009; Arany et al. 2003; Baroni et al. 2003; Basu et al. 2000; Chambaut-Guérin et al. 2000; Chou et al. 2000; Dufner-Beattie et al. 2001; Kim et al. 2009; Kini et al. 2001; Lee et al. 2008; Sharow et al. 2012; Song & Xu 2001; Wu et al. 2009; Ni et al. 2019; Ying et al. 2011).

For instance, the all-trans retinoic acid (ATRA) has been shown to help in differentiating blast cells in haemtological malignancies (Hassan HT & Rees J 1990), decreasing the potential for neoplastic proliferation and playing an important role in cell differentiation, apoptosis and adhesion (Herreros-Villanueva et al. 2015; Voigt et al. 2000). Furthermore, it has been shown that retinoic acid can suppress the gene transcription of oncogenic factors and promote the antiproliferative effect (Arnold et al. 1994), has anti-angiogenic action (Majewski et al. 1994). Besides, retinoic acid and temozolomide arrested cell cycle progression in the G0/G1 phase and significantly induced apoptosis of human glioma cells (Shi et al. 2017).

Furthermore, the effect of retinoic acid on gCSCs has been established. gCSCs are known to be the tumor initiator and tumor propagator (Wang & Liu 2019; Songthaveesin et al. 2018). It has been established
that glioma stem cells divide symmetrically, resulting in the poor therapeutic effect of current glioma treatments. If gCSCs are divided asymmetrically, the proliferative capacity of the tumor containing such cells is decreased, and if asymmetry is decreased in normal stem cells, the neoplastic transformation increases (Wang & Liu 2019). In that sense, it has been shown that ATRA induce asymmetric gCSCs cell division in U87MG glioblastoma cell line. These results suggest a therapeutic effect of ATRA on glioma stem cells (Wang & Liu 2019; Songthaveesin et al. 2018).


**Vitamin C**
Furthermore, glucosamine has been documented that glucosamine has anti-inflammatory activity via regulating inhibiting nuclear factor κB (NF-κB) action and therefore suppresses inflammatory cytokines production (Dalirfardouei et al. 2016). Hence, it may be used in treating inflammatory-induced tumors (Al-Hanbali et al. 2009; Mansor et al. 2018).

Although D-glucosamine is safe, some studies have presented the toxic effect of its conjugates on cancer cells and have highlighted its application in targeting glioma. The study revealed a significant effect on cytotoxicity and apoptosis in vitro as assessed on resistant grade IV glioma cell lines. Furthermore, this effect was not observed on normal human erythrocytes in the haemolysis test. GC liposomes were not toxic to normal brain tissues of healthy Sprague-Dawley rats tested. The absence of histological and behavioral changes together with the absence of caspase-3 in the brain tissue confirmed the suitability of the system for direct infusion into the brain (Yadav et al. 2019).

The expression of vitamin D receptors in glioma tumors is associated with increased survival (Cataldi et al. 2020; Norlin 2020). The 1,25-hydroxyvitamin D3 has direct effects on nervous systems, influencing steroidogenic pathways. In the human glioma, Vitamin D3 stimulates the expression of aromatase and the 3β-hydroxysteroid dehydrogenase as well as the 17-hydroxylase/steroid lyase in the astrocytes, in addition to providing neuroprotection. The expression of vitamin D receptors in glioma is associated with a higher survival, and 1,25 hydroxyvitamin D3 and its analogus suppress the proliferation and the migration in glioma cellular lines, expressing human vitamin D receptors (Norlin 2020).

THE IMPORTANCE OF COMBINATIONS

Although there are rationales for using each molecule in DBM, the synergistic effect of the combination makes it more powerful. For instance, the synergistic effect of MLT and VPA enhances the cytotoxic effect of TMZ in GBM cells through the reduction of MGMT expression (Di Bella et al. 2013a; Di Bella et al. 2013b; Lissoni et al. 1996; Rudà et al. 2016), and therefore increasing life expectancy as seen in our patients (Zhang et al. 2016). Furthermore, the combination of CAH inhibitors with chemotherapeutic treatments for glioblastoma increased survival.

We noticed that the integration of AAZ in a multi-therapeutic context contributed to reducing cortisone administration due to its diuretic and anti-edema properties. Besides, administrating AAZ and VPA reduced epileptic episodes and improved the prognosis.

Interaction between MLT and other DBM molecules opposes several processes characterizing neoplastic phenotype, mutation and proliferation, progression and/or dissemination. All these features suggest the use of this molecule as a treatment for cancer (Di Bella et al. 2017; Gil-Martín et al. 2019; Nooshinfar et al. 2017; Talib 2018).

Many research papers confirm the preventive and therapeutic activity of vitamin D in neoplastic pathologies (Jeon & Shin 2018). Various synthetic analogues of Vitamin D, called deltanoids, are designed specifically to potentiate antiblastic activity, and at the same time reducing several collateral effects (Negri et al. 2020). Synergism of Vitamin D, Vitamin C, Vitamin E, and MLT was found in several clinical studies, showing their antiproliferative, antiangiogenic, and differentiative properties.

Furthermore, we hypothesize that the combination of Calcitriol and ATRA (All-Trans Retinoic Acid) and Temozolomide could be a safer approach to benefit from vitamin D in high degree glioma cancer management. The addition of acetazolamide to this protocol can reduce the risk of brain pseudotumors, because of vitamin D and a surplus of vitamin A can lead to intracranial hypertension; this approach can provide benefits due to antitumoral activity of acetazolamide (Elmaci et al. 2019).

TREATED PATIENTS WITH MALIGNANT BRAIN TUMORS

The Patient's age, diagnosis, and initial treatment used before DBM are summarized in Table 2. Below is a briefing about each case and the follow-up results after DBM.

Patient ID: 5833
Date of birth: 29/07/1989
DIAGNOSIS 20/06/2012 (23 years old) ANAPLASTIC ASTROCYTOMA (WHO III) unmethylated-MGMT; mutated IDH1; EGFRvIII negative; overexpressed VEGF.

20/06/2012 – SURGICAL RESECTION (bilobed space-occupying lesion, around 4 cm)
26/07/2012 – Brain MRI: “...small residue of the most caudal part of the left parietal glial lesion...”
30/07/2012 – RT + TMZ 135 mg (up to 06/11/2012)
21/11/2012 – TEM 370 mg/12 courses
27/12/2012 – PET: “...hypodense nodular area, caudally to the surgical cavity, in the left parietal region...”
05/03/2013 – Brain MRI: “...disease residue along the edges of the seemingly stable surgical cavity, even though the rCBV values are increased compared to the previous scan...”
05/10/2013 – Brain MRI: “...further signal alteration located in the left parietal corona radiata, suspected to be mild infiltrative disease progression...”
05/11/2013 – START DBM
20/06/2014 – Brain MRI: “...morphology and extension of the infiltrative disease residue along the lower edge of the surgical cavity are unaltered... the small area of signal alteration located in the left parietal corona radiata is also unaltered”
Tab. 2. Patient’s age, diagnosis, and treatments before initiation of DBM

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (y) at the time of diagnosis</th>
<th>Time of diagnosis</th>
<th>Diagnosis</th>
<th>Mutation/Expression</th>
<th>Therapy after diagnosis</th>
<th>Initiation of DBM</th>
<th>Survival after DBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>5833</td>
<td>23</td>
<td>Jun 2012</td>
<td>Anaplastic astrocytoma, (WHO III)</td>
<td>unmethylated-MGMT; mutated IDH1; EGFRvIII negative; overexpressed VEGF</td>
<td>Surgery + Chemotherapy + Radiotherapy</td>
<td>Nov 2013</td>
<td>&gt; 7 years</td>
</tr>
<tr>
<td>6245</td>
<td>23</td>
<td>May 2007</td>
<td>Oligoastrocytoma (II)</td>
<td>unmutated IDH1; mutated IDH2; methylated-MGMT; 1p/19q codeletion</td>
<td>Surgeries + Chemotherapy + Radiotherapy</td>
<td>Nov 2014</td>
<td>&gt; 6 years</td>
</tr>
<tr>
<td>6572</td>
<td>27</td>
<td>Feb 2015</td>
<td>Anaplastic astrocytoma (III)</td>
<td>Mutated-IDH1; methylated-MGMT; GFAP positive</td>
<td>Surgery + Chemotherapy</td>
<td>Mar 2015</td>
<td>&gt; 5 years</td>
</tr>
<tr>
<td>9691</td>
<td>19</td>
<td>Nov 2010</td>
<td>Oligoastrocytoma (II)</td>
<td>unmutated IDH1; IP53 negative; GFAP positive; NOGO-A positive</td>
<td>Surgeries</td>
<td>Aug 2014</td>
<td>&gt; 6 years</td>
</tr>
<tr>
<td>4835</td>
<td>40</td>
<td>Nov 2012</td>
<td>GBM (IV)</td>
<td>mutated IDH1</td>
<td>Surgery</td>
<td>Dec 2012</td>
<td>&gt; 8 years</td>
</tr>
<tr>
<td>5371</td>
<td>49</td>
<td>May 2013</td>
<td>Anaplastic glioma (III)</td>
<td>ND</td>
<td>-</td>
<td>Sept 2013</td>
<td>&gt; 7 years</td>
</tr>
<tr>
<td>5251</td>
<td>43</td>
<td>Nov 2012</td>
<td>Oligodendroglioma (?)</td>
<td>ND</td>
<td>-</td>
<td>Apr 2013</td>
<td>&gt; 7 years</td>
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25/06/2014 – PET: “...reduction in radio-compound uptake at the hypodense nodular area, previously reported caudally to the surgical cavity in the left parietal region...”

29/12/2017 – PET: “...the PET-CT scan has not shown any amino acid tracer distribution abnormalities that could be related with certainty to a neoplastic disease...”

23/03/2018 – Brain MRI: “...morphology and size of the surgical cavity are unaltered... the areas of signal alteration are also unaltered, T2/FLAIR hyperintensities on the edges of the cavity, without contrast agent uptake. No documented increase in rCBV values at these alterations...”

18/10/2018 – MRI: Unaltered diagnosis

10/06/2019 – MRI: Stable disease diagnosis with unaltered main MRI findings

10/01/2020 – PET-CT: “...has not shown any amino acid tracer distribution abnormalities that could be related to a neoplastic disease, particularly at the edges of the surgical resection in the left parietal region. No further areas of focal uptake of 11C-Methionine at cortical and subcortical regions and in the cerebral hemispheres.”

26/06/2020 – Brain MRI: Stable disease diagnosis with unaltered main MRI reports.

26/06/2020 – Brain MRI with Contrast Agent: “...shows non-significant increase in perfusion in some ROIs and choline peaks in some voxels on spectroscopy. No significant enhancement after contrast agent. Ended due to the absence of recurrent disease.”

10/10/2020 – Histological exam: “Fragment of squamous epithelioma in situ (pTis UICC 2017)”

Patient ID: 6245
Date of birth: 04/12/1982
DIAGNOSIS 21/05/2007 (25 years old)
OLIGOASTROCYTOMA (WHO II) unmutated IDH1; mutated IDH2; methylated-MGMT; 1p/19q codeletion

21/05/2007 – 1st SURGICAL RESECTION
31/05/2013 – 2nd SURGICAL RESECTION (1st recurrence)
21/10/2014 – 3rd SURGICAL RESECTION (2nd recurrence) – RX + TEM 75 mg
25/11/2014 – START DBM

20/07/2015 – Brain MRI: “...no pathological alterations shown in the sequences performed after Gadolinium administration...”

30/03/2016 – Brain MRI: “...findings essentially unaltered...”

21/11/2017 – Brain MRI: “...findings essentially unaltered...”

02/05/2018 – Brain MRI: “...findings essentially unaltered...”

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27/11/2018 – Brain MRI: “...findings essentially unaltered...”
22/05/2019 – Brain MRI: “stable neuradiological diagnosis compared to previous”
12/05/2020 – Brain and brain stem MRI: Stable diagnosis
13/05/2020 – Oncology Visit: “In light of the recent instrumental reassessment that documented significant disease stability, and optimal and stable general clinical conditions, it is recommended to keep monitoring the patient”
18/11/2020 – Brain and brain stem MRI: “Today’s findings appear completely unaltered. [...] All the remaining findings are essentially unaltered.”

Patient ID: 6572
Date of birth: 21/02/1988
DIAGNOSIS 02/02/2015 (27 years old) ANAPLASTIC ASTROCYTOMA (WHO III)
Mutated-IDH1; methylated-MGMT; GFAP positive

16/01/2015 – SURGICAL RESECTION (no CT/2 RT sessions)
27/02/2015 – Brain MRI: “...centimeter residual area of T1 hyperintensities and T2 hypointensities is observed with a diffuse oedema. Adjacent to the previous finding, millimetric area of T1 hyperintensities as well as the enhanced area after contrast agent...”

Subsequently SEIZURES – PROGRESSIVELY WORSENING HEADACHE – PARTIAL STATUS EPILEPTICUS
NEW SURGERY PROPOSED followed by RT – TMZ
16/03/2015 – START DBM
20/04/2016 – Brain MRI: “...slightly less evident, the small area of signal alteration refers to the residual lesion after contrast agent...”
03/10/2017 – Brain MRI: “...no pathological enhancement after administration of the contrast agent...”
02/07/2018 – Brain MRI: “...No areas of pathological enhancement attributed to residues/recurrences are shown...”
06/02/2019 – Brain MRI: “The findings are essentially unaltered... no significant areas of pathological enhancement are observed”
09/10/2019 – Brain MRI: “...compared to the previous scan on 30/01/19: findings unaltered; left frontal region with an area of T2 hyperintensities. Frontal horn of left lateral ventricle remains wide. Diffusion and perfusion imaging do not show areas of ADC reduction and rCBV increase at the aforementioned area of T2 hyperintensities. Remaining findings are unaltered, particularly in the malacic area affecting the right cerebral hemisphere and left thalamic ischaemic cavity, as well as the likely nodule of grey matter heterotopia along the wall of the right lateral ventricle chamber. No areas of pathological enhancement after contrast agent.”
15/07/2020 – Brain MRI: “Compared to the exam observed on 09/10/2019, the baseline and post-contrast enhancement MRI findings are perfectly comparable. Currently no images are observed that are consistent with neoplastic recurrence.”

Patient ID: 9691
Date of birth: 20/11/1991
DIAGNOSIS: 30/11/2010 (19 years old) OLIGOASTROCYTOMA (WHO II) unmutated IDH1; IP53 negative; GFAP positive; NOGO-A positive

16/11/2010 - 1st SURGICAL RESECTION (5 cm mass)
15/02/2013 – 2nd SURGICAL RESECTION (recurrence) – diagnosis: OLIGODENDROGLIOMA WHO II
10/06/2013 – Brain MRI: “...residual lesion...”
08/07/2014 – Brain MRI: “...extension of the residual lesion is unaltered, but diffusion imaging shows a 2-fold ADC increase compared to the white matter, and perfusion also shows an rCBV value that is 5 times that of the reference white matter...”

JUDGED TO BE INOPERABLE (RIGHT FOOT PARALYSIS – DEPRESSION – CLONUS)
08/08/2014 – First revision: Prof. Giangaspero – Umberto I – Policlinico di Roma [Umberto I Polyclinic of Rome]: GRADE 3 ANAPLASTIC ASTROCYTOMA
18/08/2014 – START DBM
28/08/2014 Brain MRI: “...slight increase in extension of the area of signal alteration, hyperintense on T2-weighted sequences, affecting the nervous tissue surrounding the surgical wound, that seems to contra-laterally infiltrate through the trunk of the corpus callosum...”
03/12/2014 – Brain MRI: “...size and characteristics of the signal in the area of T2 hyperintensities are essentially unaltered...”
03/06/2015 – Brain MRI: “...the area of T2 hyperintensities is essentially comparable... diffusion imaging shows this at the area of signal alteration. Increased ADC values (2.6) compared to white matter (1); perfusion imaging does not detect significantly increased rCBV values”
09/09/2015 – Brain MRI: “...no areas of pathological contrast enhancement are observed after administration of the contrast agent...”
22/11/2016 – Brain MRI: “...findings unaltered... diffusion and perfusion imaging do not show areas of ADC reduction or rCBV increase...”
28/03/2017 – Brain MRI: “...the diagnosis remains stable...”
01/09/2017 – PET: “no areas of significant uptake at the known lesion, outlined in the MRI scan on 29/03/2017,
in the left parasagittal posterior frontal hemisphere...the scan did not show the presence of areas of pathological radiopharmaceutical uptake that could be related to the primary disease..."

The patient reduced the doses of the medicinal product on their own initiative, followed by some seizures and worsening of general conditions.

31/07/2018 – Brain MRI: "...stable radiological findings...no apparent recurrence of the known space-occupying lesion from their medical history..."

Resumption of somatostatin intravenous infusion treatment at full doses, resolution of seizures

09/07/2020 – Brain MRI: "Compared to the previous MRI on 20/02/2020, the onset of an area of pathological contrast enhancement in the left parietal intraventricular region is observed, likely neoplastic with irregular morphology and around 20 mm in diameter. Perfusion imaging shows rCBV values that are increased 3-fold at this area of pathological enhancement compared to those of the contralateral white matter, likely in relation to signs of neoangiogenesis. A small perilesional oedema is associated with this area of pathological enhancement. Increased extension of the known area of left high-frontal signal alteration [...]posterosuperiorly to the known surgical cavity, rCBV values that are increased 2/3-fold are observed compared to those of the contralateral white matter. Compared to the abovementioned previous MRI scan, the onset of multiple small and markedly hypointense areas are observed at the same area on the T2*-weighted GE images, such as the presence of haemosiderin, suspected to be multiple small..."

09/11/2015 – MRI: "...the radiological findings appear to be essentially unaltered... in particular, the known space-occupying lesion located at the cingulate gyrus, which affects both hemispheres, is still significant... “...no areas of intra- or extraparenchymal pathological enhancement are observed at either the lesion or the remaining sub- and supratentorial parenchyma after administration of the contrast agent...”

29/04/2016 – MRI: "...the sizes and signal characteristics of the known space-occupying lesion located at the cingulate gyrus, which affects both hemispheres, remains essentially unaltered...”

02/11/2016 – MRI: "...during today's scan, after administration of the contrast agent, the abovementioned poorly-defined area of contrast enhancement at the lesion in the right paramedian region, appears to be less apparent...”

15/02/2018 – MRI: "...no signs of locoregional recurrence are detected as a result of removing the glioblastoma that was previously located at the cingulate gyrus with bihemispheric involvement...”

29/05/2019- MRI: "...the onset of signs of locoregional disease recurrence are not observed...Remaining findings are unaltered...”

19/03/2020 – Brain MRI: “absence of enhancement attributed to signs of disease recurrence. Diffuse leukomalacia persists at the centra semiovale and in the periventricular region, unaltered compared to the previous scan. Sub- and supratentorial ventricular system of normal morphology, volume and location. Slight enlargement of the vault fluid spaces. Developmental venous anomaly in the left cerebellar region. No intra- and extra-axial pathological enhancement detected after contrast agent. Mucosal thickening of left maxillary sinus. Turbinate hypertrophy.”

Patient ID: 5371

Date of birth: 22/06/1964
Di Bella et al: A retrospective observational study on cases of anaplastic brain tumors treated with the Di Bella Method

Diagnosis: 06/05/2013 (49 years old) Progressive anaplastic glial tumour, grade III infiltrating glioma
Molecular analysis on mutations has never been performed

06/05/2013 – MRI: “...extensive cerebral tumor involving the entire bulbo-medullary junction with diffusion to the inferior cerebellar peduncles, right middle cerebellar peduncle, and infiltration of the 4th ventricle on which it already determines the mass effect...”

10/09/2013 – START DBM
“Progressive anaplastic glioma, grade III infiltrating glioma” Diagnosis, exacerbated by the highest functional dignity of the affected area, crossed by 10 cranial nerves, from the 3rd to 12th (including the optic and olfactory nerves) and the 4th ventricle, site of the vital nerve, respiratory and cardiovascular centers that can be easily made inactive by compression (for tumor progression, and/or postoperative or post-therapeutic cerebral edema). The location and extension caused paresis and disability. A court-appointed Technical Advisor, who the patient had asked to get DBM, excluded radiotherapy, operation, and confirmed the possibility of palliative treatment with a prognosis of less than a year.

DBM achieved remission in the majority of the tumor and stability, blocking progression of the residual tumor cells that are no longer proliferating, finally enabling functional recovery and self-sufficiency in a patient that was previously known for disability and assistance.

29/01/2019 – Brain MRI: “the extension of the neoplastic infiltration detected at the bulbocervical region remains stable, bilaterally with an increase in volume of the anatomic structures in question and inhomogeneous signal alteration”.

17/04/2019 – FDG-PET: “compared to the MRI on 29/01/2019: the presence of inhomogeneous metabolic tracer uptake is detected, with various degrees of mild to moderate uptake at the alteration detected on the MRI. The uptake is reported to be more accentuated on the right-hand side. Moreover, moderate-severe uptake is detected at the right column, of uncertain significance (aspecific? Other?); useful assessment in characterized field. There is nothing else to report in the remaining examined body regions, particularly in the lung parenchyma and the liver. The PET findings suggest the presence of heteroplastic tissue with moderate carbohydrate metabolism at the bulbocervical junction and proximal segment of the cervical cord. N.B. in the hypermetabolic region, the activity is defined as mild-moderate-severe compared to the hepatic metabolic activity, pursuant to EANM guidelines.”

Patient ID: 5251
Date of birth: 06/03/1969
Diagnosis: November 2012 (43 years old)
Molecular analysis on mutations has never been performed

17/12/2012 – CT ANGIOGRAM and MRI: show a tumor with “progressive tendency” (compared with an MRI from 2006 in which it is barely noticeable, so much so that it was not taken into account at the time), with severe vascularization and sticking closely to the sylvian artery, therefore “surgery is not recommended”. Considering the progressive characteristics and the spectroscopy and perfusion imaging findings, the diagnostic hypothesis puts Glioblastoma or Oligodendroglioma or Oligoastrocytoma.

19/04/2013 – START DBM
20/03/2014 – MRI: “...the presence of a space-occupying lesion, 1.95 x 1.59 cm in size, is confirmed... with mild enhancement after contrast agent...”

20/03/2014 – PET: “...the lesion, composed of solid and calcified portions, has no substantive changes compared to what was previously documented...”

29/01/2019 – MRI: “...the scan has not shown the evolution of the left temporosylvian lesion...”

27/10/2020 – Brain MRI, perfusion imaging and spectroscopy: “The left temporosylvian lesion remains completely unaltered, with extensive calcified parts and a smaller parenchymatous part.”

Outcomes of DBM protocol
Three of the seven patients treated with DBM are still alive after 5 to 6 years, and the other four patients are still alive after seven years of starting DBM (Table 2). In 3 cases (IDs 5833, 6245, 6572), DBM began after the failure of Stupp protocol, i.e., when disease recurrence was detected. After DBM treatment, disease progression was arrested.

The patient with GBM (ID 4835) had incomplete surgical resection then adapted DBM as first-line therapy a month later. After eight years of DBM, the patient is still alive and has neither symptoms nor disease progression. Furthermore, patient 5371, who had inoperable aplastic glial neoplasm received neither surgery nor Stupp protocol, started DBM as first-line therapy during tumor progression. After seven years, the patient did not develop recrudescence and is cancer-free. Also, patient 5251, with inoperable oligoastrocytoma, didn’t start Stupp therapy and didn’t have surgery. Eight years following DBM, the patient is still alive after eight years with free-tumor progression and living a normal life.

Discussion
In malignant brain tumors and GBM, the prognosis remains poor despite surgery, chemo- and radiotherapy, with a median survival of 14-18 months after diagnosis.
In our study, DBM improved life expectancy and quality of life and avoided relevant toxicity (Di Bella et al. 2017; Di Bella et al. 2013a; Di Bella et al. 2013b; Di Bella 2005; Di Bella 2019; Di Bella 2010; Di Bella et al. 2018; Di Bella et al. 1979a; Di Bella et al. 1979b; Di Bella 1997; Di Bella & Di Bella 2015; Di Bella & Gualano 2006; Di Bella & Di Bella 1998).

During most tumors' progression, with more evidence in brain ones, the percentage of CSCs compared with other cellular components of the neoplastic population is high, and it's associated with chemo and radiotherapy resistance and the rapid progression of the disease. For this reason, we have gradually increased the dosage of molecules that negatively affect CSCs and reprograms CSCs.

Unlike Stupp's protocol, the metronomic administration in DBM of temozolomide (20 mg in the morning and the evening) and hydroxyurea (500 mg at lunch) has enhanced proliferation control and invasiveness of cancer cells. Besides, the daily administration of MLT (100mg) with the retinoid solution in vitamin E reduced the myelotoxicity of temozolomide and hydroxyurea (Di Bella et al. 2013a; Di Bella et al. 2013b; Di Bella & Di Bella 2015; Di Bella & Gualano 2006).

The DBM, in contrast with a classical oncological point of view, moves the therapeutic axis from cytolytic, toxic, and immunosuppressive mechanisms to the contrast of negatively regulating oncogenesis through different mechanisms (Di Bella et al. 2018; Perry et al. 2008). Furthermore, differentiating components of DBM, such as a retinoid solution in vitamin E, vitamins C, D, and MLT, counteract the mutagenic capability of tumor cells, based on a defense system and a survival program that allows efficient and rapid repair of DNA damages induced by chemo- and radiotherapy. For instance, the prokaryotes, the first forms of life, are still surviving until the present day. Thanks to a defense system developed during evolution based on a mutational program, DNA repair in case of adverse events. The prokaryotes survival program termed the state of emergency, “SOS program,” has been passed to the somatic cells (Radman 1975). In addition, it has been shown that the SOS program is activated in tumor cells, and many homologies in neoplastic cell genes and bacterial ones have been identified (Israel 1996).

Cancer cells in acute stress implement DNA repair systems and express or silence genes according to their needs, selecting and retaining for each mutation a whole series of advantages with speed and efficiency far superior to bacterial cells. The SOS system allows the neoplastic population to progressively become insensitive to different oncotherapeutic drugs through DNA repair and recombination.

The SOS system is silenced and inactivated in stable biological conditions, transcriptionally stopped by a repressor, the LEX-A protein. When severe damages occur in a somatic cell DNA, the transcriptional repressor LEX-A is turned off by the positive regulator REC-A. The SOS activation carries along with a series of mutations that repair but, at the same time, modify the DNA, leading to carcinogenesis. The mutating cells start a progression of the SOS program, in which a continuous selection and retention of mutations confers a series of advantages, as confirmed by (Lambert et al. 2011) and recently by (Russo et al. 2019).

In conclusion, the multi-strategic objectives of DBM are inhibiting the proliferative-invasiveness and neoplastic angiogenesis, and silencing the SOS survival system through differentiated components, like the retinoid solution in vitamin E, MLT, vitamin C, and D, etc. Besides, the differentiative components of DBM display trophic, immunomodulatory, and antioxidant activities, improve vitality and efficiency of normal cells and depress the efficiency and vitality of neoplastic ones.

The DBM is expanding its activity to numerous vital responses typical of neoplastic biology. DBM moves the therapeutic axis from a merely cytotoxic and cytolytic activity that expected a utopian and elusive lifelong eradication of all tumor cells in the body to an immuno-neuro-endocrine homeostasis recovery. A more physiologic strategy thought the reversion of vital functions that deviate in cancer cells, the differentiation of tumor cells, and the reprogramming of cancer stem cells.

REFERENCES


Di Bella L, Di Bella G (2015). Solution of retinoids in vitamin E in Travel factory. [(Cancer: Are we on the right track?) (In Italian)].


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Documentation of the numerous and serious anomalies that have deprived the ministerial experimentation of the 1998 DBM of any scientific dignity:

Marcus Müller (1999). Di Bella’s therapy the last word? BMJ: 318:208-209. “The authors also say that they could not have done randomised trials for ethical reasons, but these are not clear. Indeed some would claim that the inferior design of their studies was unethical.”

Müllner M (1999). Di Bella’s therapy: the last word? The evidence would be stronger if the researchers had randomised their studies. BMJ. Jan 23;318(7178):208-9. doi: 10.1136/bmj.318.7178.208


