Glioblastoma multiforme (GBM) is the most common malignant primary CNS tumor with two to three cases per 100,000 inhabitants per year in Europe or North America. The standard treatment consists of gross total resection followed by focal irradiation to the tumor bed with concomitant and adjuvant temozolomide (TMZ). Despite a decade of intensive research and a variety of chemoradiotherapy regimens investigated, the outcome of patients with GBM has not changed significantly with a reported median survival of 15–18 months [1,2].

The key role of GBM chemoresistance seems to be played by tumor stem cell with its unlimited proliferative potential. GBM stem cells have shown resistance both to clinically used chemotherapies and radiotherapy [3]. In order to achieve the complete eradication of the tumor, GBM progenitors would be the mainly therapeutic target [4].

In the past decades, there have been a variety of trials conducted in GBM patients utilizing different chemotherapeutic agents in combination with focal radiotherapy. All have shown depressing survival results. In 2005, the addition of TMZ, an oral alkylating agent, to standard radiotherapy resulted in a clinically meaningful and statistically significant outcome benefit with slight additional toxicity as compared with radiation alone in newly diagnosed GBM [5].

More recently, a clinical trial evaluated the association of valproic acid and TMZ during radiotherapy and survival of GBM. In a multivariable Cox regression analysis, use of valproic acid was significantly associated with longer survival [6].

The role of anthracycline in gliomas treatment
Doxorubicin (Dox), an anthracycline antibiotic which works by intercalating DNA and inhibiting topoisomerase II, is commonly used in therapy of various tumors, including hematological malignancies, carcinomas and soft tissue sarcomas. The therapeutic efficacy of Dox is reduced in CNS tumors because of its poor penetration through the blood–brain barrier (BBB) mediated by MDR efflux transporters.

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Although Dox is a very effective anticancer drug, its cardiotoxicity has to be considered as a potential limitation to unrestricted use [7]. Dox is one of the best-studied drugs for high-grade gliomas in preclinical trials. In vitro and in vivo studies suggested that Dox had a strong antineoplastic activity against human gliomas. Dox, when delivered locally, presented an in vivo intracraniac activity in an orthotopic xenograft model of 9L gliosarcoma cells. Rats treated with Dox polymers had significantly extended survival. The median survival for the control group was 21 days compared with 34 days (p < 0.01) for the 3% Dox group and 45 days (p < 0.0001) for the 5% Dox group. Thus, local Dox treatment appears to be active in a dose dependent [8].

In order to evaluate the chemosensitivity of primary cultures of pediatric malignant gliomas, a chemical analysis was considered, which included 21 consolidated anticancer agents and new agents as small molecules and monoclonal antibodies. Cell death was assessed and the survival percentage was calculated for each compound tested in the assay. Data obtained from this study verified that among the classical chemotherapeutic drugs only Dox, mitoxantrone and melphalan most induced significant cell death in primary glioma cultures [9].

Moreover, GBM cell lines have also been used to explore the cytotoxic and proapoptotic effects of Dox. In vitro studies have been carried out in order to assess the cytotoxic effect of Dox on U87MG and A172 GBM cell lines. These cell lines were treated for 24 and 48 h with different doses of Dox (0.1–0.5 µg/ml); after 24 h of exposure, the data have displayed that there was no difference in cell viability comparing U87MG-treated cells versus untreated control, while A172 has presented a statistically significant difference in cell viability comparing treated cells versus untreated control. In contrast, after 48 h of exposure, both A172 and U87MG cell lines have showed a significant statistically difference in cell viability compare to untreated controls, confirming that U87MG and A172 cell lines are very sensitive to Dox exposure [10].

There is overwhelming evidence that GBM CD133+ cells with their unlimited proliferative potential seem to be the only tumorigenic population [11].

Eramo et al. studied the effect on cell survival of undifferentiated GBM cells treated with commonly used antineoplastic drugs and anthracyclines at dose range based on in vitro IC50 in malignant gliomas. After 48 h of treatment, a marked resistance of GBM stem cells was observed to all the used compounds, whereas Jurkat cells and erythroblasts used as control displayed high rates of cell death. In addition, it was also investigated the ability of GBM stem cells to recover and proliferate following the chemical exposure. Cells were treated with chemotherapeutic agents for 24 h, thus the cytotoxic stimuli were removed and the cells number was evaluated after 2 weeks. GBM stem cells were able to recover and proliferate than untreated control while chemosensitive tumorigenic stem cells isolated from small cell lung cancer exhibit a limited recovery. These results confirm the natural history of the disease progression in GBM patients, which are poor responder to antineoplastic treatments, invariably followed by tumor recurrence. Intriguingly, only after prolonged exposure (96 h) to anthracyclines, daunorubicin and Dox, GBM stem cells displayed an higher apoptotic rate while the commonly used TMZ was not quite effective [12].

Similarly, our recent study on T98G Dox-resistance GBM cell line demonstrated an increased cytotoxicity after 96 h exposure to Dox while shorter exposures (24–72 h) did not show any statistically differences compare to ‘nontreated’ controls [10].

Influence of radiation on the BBB
It is noteworthy that the irradiation may alter the structure and permeability of the BBB. Whole-brain radiation causes an intense vesicular reaction of the cortical microvascular endothelium without the opening of the tight junctions. Standard doses of CNS irradiation have been described to cause an inflammatory response in mouse brain tissue through the activation of astrocytes and microglial cells [13,14]. The irradiation of normal brain tissue causes a damage of the BBB with a linear relation with radiation dose. CNS tissue that was not irradiated presented no altered function of the BBB by 99mTC GH imaging. Interestingly, the BBB permeability in and around the malignant brain tumor was approximately 20%. After a total dose of 30 Gy irradiation the permeability got to 75%. 8 months after irradiation, BBB permeability in the tumor had returned to pretreatment levels and in normal tissue the BBB recovered entirely [15].

Thus, radiation could induce well-defined changes in BBB function and increase the permeability to antineoplastic agents [16]. Activated
Permeability of the brain accompanied by an increase in BBB microvascular response to fractionated irradiation of fractionated period). They documented that persisted throughout the experiment. No response 60 days from the start of irradiation 6 Weller M, Gorlia T, Cairncross JG.

5 Stupp R, Mason WP, van den Bent MJ.


