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Ultrafractionated Radiation Therapy (3 Daily Doses of 0.75 Gy) - A New and Promising Radiotherapy Schedule for Glioblastoma Patients

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1. Introduction

Malignant glioma is one of the most radio-resistant tumor types and accounts for approximately 60% of all primary brain tumors in adults (Behin et al., 2003; Black, 1991a, 1991b; DeAngelis, 2001). There are three distinct histological types: anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), and glioblastoma multiforme (GBM). The prognosis of malignant glioma patients remains dismal (Behin et al., 2003; Black, 1991a, 1991b; De Angelis, 2001). The median survival for patients with newly diagnosed GBM is 8 to 15 months, prognosis is slightly better for newly diagnosed AA with a median survival of 24 to 36 months, and the prognosis for AO gives a median survival of 60 months (Behin et al., 2003; Black, 1991a, 1991b; De Angelis, 2001). For AA and GBM, the standard of care consists of surgical resection of as much of the tumor as is considered to be safe, followed by radiation and chemotherapy and has been so for many decades (Behin et al., 2003; Black, 1991a, 1991b; DeAngelis, 2001, Fine et al., 1993; Stewart, 2002; Walker et al., 1978, 1980). A new standard procedure for GBM has recently been defined by the EORTC phase III trial which randomized patients in two groups, receiving either temozolomide (TMZ) concomitant and adjuvant to radiation therapy or radiation therapy alone (Stupp et al., 2005). A significant increase in overall survival (OS) was seen in the radiation therapy plus TMZ group compared to the radiation therapy alone group. Survival rates were respectively 14.6 and 12.1 months. For AO, the standard treatment is surgical resection followed by radiation therapy (Stupp et al., 2005). Adjuvant chemotherapy does not provide significant benefits in OS (Van den Bent et al., 2006).

Radiation therapy remains the backbone of care for glioblastomas, even in patients who have undergone a prior presumed complete resection. The infiltrative nature of these tumors makes a truly complete resection nearly impossible in most cases (Behin et al., 2003; Black, 1991a, 1991b; DeAngelis, 2001, Fine et al., 1993; Hall, 1978; Stewart, 2002; Walker et al., 1978, 1980). Standard fractionated radiation therapy delivers a total radiation dose of 60 Gy given in 30 fractions over 6 weeks. The target is usually the tumor bulk as visualized on CT or MRI, with a wide margin of 2-3 cm (Behin et al., 2003; Black, 1991a, 1991b; DeAngelis,

2001, Fine et al., 1993; Hall, 1978; Stewart, 2002; Walker et al., 1978, 1980). Although radiation therapy is not a curative treatment for glioblastomas, it results in prolongation of life with optimized quality (Behin et al., 2003; Black, 1991a, 1991b; DeAngelis, 2001, Fine et al., 1993; Stewart, 2002; Walker et al., 1978, 1980). Whether the clinical radioresistance of GBM is due solely to inherent radioresistance at the cellular level is unclear. Overall, malignant glioma cell lines exhibit SF2 (SF - 2 Gy) values at the upper end of the range compared with other human tumor cell lines, though studies have failed to link clinical response with SF2 (Taghian, 1992, 1993). Defining the molecular basis of radioresistance is, therefore, important. Disruption of cell-cycle arrest or apoptotic pathways by *INK4a* loss or by *p53* mutations or inactivation (approx. 40–60% of malignant gliomas have *p53* mutations) associated with *CDK4* amplification or *Rb* loss may be significant factors in determining the response of these tumors to irradiation and treatment outcome (James & Olson, 1986; Kleihues & Ohgaki, 1999, Watanabe et al., 1996).

The radiation survival response of mammalian cells is more complicated than once believed. A few studies indicate that some human cell lines are sensitive to killing by low radiation doses (1 Gy). This has been termed *low-dose hyper-radiosensitivity* (HRS) (Joiner et al., 1986; Lambin et al., 1994b, 1996; Marples et al., 1997; Short et al., 1999b; Turesson & Joiner, 1996). This phenomenon is more apparent in radioresistant cell lines such as glioma cells, and is substantially underestimated by the linear-quadratic (LQ) model (Joiner et al., 1986; Lambin et al., 1994b, 1996; Marples et al., 1997; Short et al., 1999b, 2001; Turesson & Joiner, 1996; Wouters et al., 1996). It may reflect differential triggering or induction of repair mechanisms. Cells may be sensitive to low doses because repair mechanisms are not induced, whereas higher doses may cause enough damage to induce or trigger repair mechanisms and, therefore, exhibit increased radioresistance (Joiner et al., 1986; Lambin et al., 1994b, 1996; Marples et al., 1997; Short et al., 1999b, 2001; Turesson & Joiner, 1996; Wouters et al., 1996). Still, new modalities of radiation therapy are urgently needed.

2. *In Vitro* studies

2.1 Cell lines experiments

The GRAY laboratory first demonstrated an increased X-ray sensitivity in murine skin and kidney after very low doses per fraction (Joiner & Denekamp, 1986; Joiner et al., 1986; Joiner & Johns, 1988). They irradiated the V79 murine fibroblast cell line with 250kVp X-rays and measured cell survival with a Dynamic Microscopic Imaging Processing Scanner (DMIPS) cell analyzer (Joiner et al., 1993a; Marples et al., 1994). Briefly, 3000-5000 cells were plated into 25 cm^2 tissue culture flasks and left to incubate for 4 to 6 hours at 37° C. The flasks were removed from the incubator halfway through the initial 4-6 hour incubation period, the medium removed, and the flasks were then immediately completely refilled with fresh medium before being sealed. Following irradiation, the DMIPS cell analyser was used to locate and record the positions of 300-400 isolated cells within 10 cm^2 in the centre of each flask. After 6-7 days of incubation at 37°C, all the originally recorded cell locations were revisited to assay for colony formation using a criterion for survival of 50 cells or more per colony as determined by manual microscopic examination of each selected location in the flask (Marples et al., 1994). The results displayed an increased X-ray sensitivity (hypersensitivity) after very small doses (< 0.3 Gy), followed by an increase in survival after the doses increased from 0.3 – 1 Gy (Joiner et al., 1993a; Marples et al., 1994). The first

phenomenon was defined and termed "low-dose hyper-radiosensitivity" (HRS), and the second phenomenon "increased radio-resistance" (IRR) (Joiner et al., 1986; Lambin et al., 1994b, 1996; Marples et al., 1997; Short et al., 1999b, 2001; Turesson & Joiner, 1996; Wouters et al., 1996). While the LQ model underestimates the HRS phenomenon, it correlates to the data at doses ranging from 2 – 5 Gy. HRS was represented as an undeniable downward "kink" on survival curve for doses below 1 Gy (Fig. 1). This was demonstrated by Wouters et al using the flow cytometry survival (FACS) method thus showing that it was not merely an artifact associated with the DMIPS assay (Wouters & Skarsgard, 1994). HRS has also been triggered in the human lung epithelial cell line, L132, after exposition to very low-doses of X-rays (Singh et al., 19974), and found with Chinese Hamster cells (Joiner et al., 1993a; Marples et al., 1994).

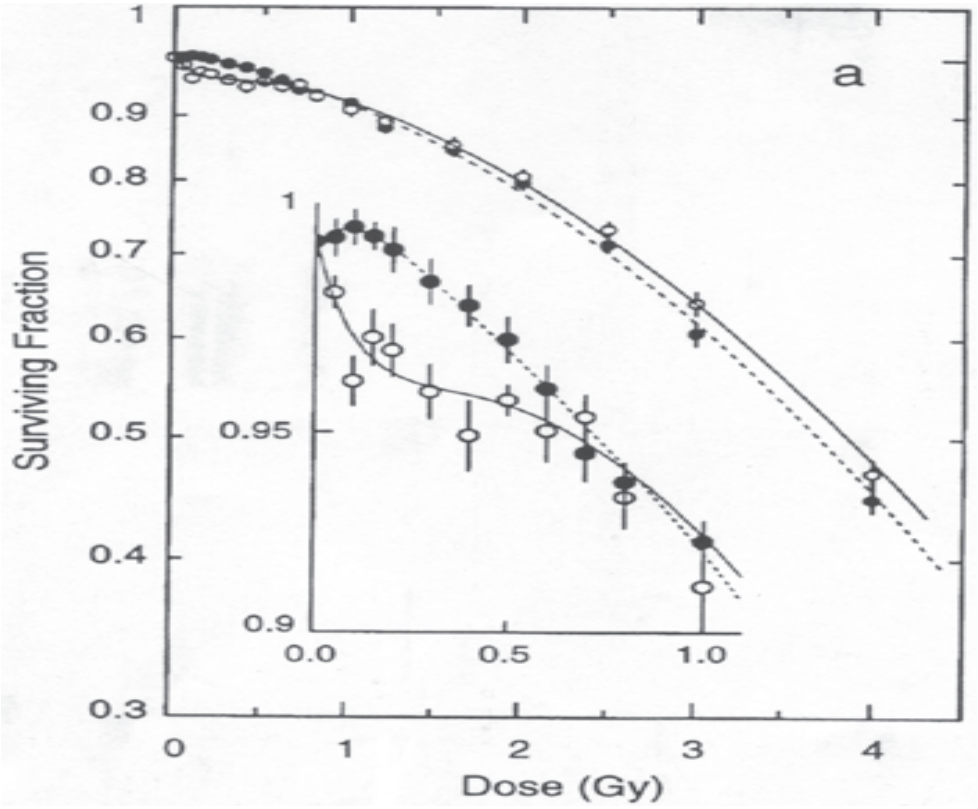


Fig. 1. Low-dose hypersensitivity was represented as an undeniable downward "kink" on survival curve for doses below 1 Gy, followed for doses superior to 2 Gy by "IRR" or "increased radio-resistance" phenomenon.

Lambin et al irradiated the HT 29 cell line, derived from a human colorectal tumor and considered as a radio-resistant tumor at usual X-ray doses, with single-doses of X-rays from 0.05 to 5 Gy. They focused on cell survival at doses of less than 1 Gy, using the DMIPS cell analyzer (Lambin et al., 1993a, 1993b). At doses < 0.5 Gy, an increased X-ray sensitivity was

observed. The HT 29 cell line was also irradiated with neutrons [d(4)-Be], obtained by a Van de Graaff accelerator bombarding a thick beryllium target with 4 MeV deuterons, at a dose rate of 0-20 Gy/min, but no HRS was observed (Lambin et al., 1993a, 1993b). In another study, Lambin et al studied an RT 112 cell line derived from a bladder carcinoma (Lambin et al., 1994a, 1994b). At a survival fraction of 60 % at 2 Gy (SF 2 Gy) this tumor was considered to be as radio-resistant (Lambin et al., 1994a, 1994b). The cell line was irradiated with low doses X-ray, and the HRS phenomenon observed at doses of < 0.5 Gy using the DMIPS method (Lambin et al., 1994a, 1994b).

The cell lines Be 11 and MeWo derived from melanoma, SW 48 from a colorectal tumor, and HX 142 from a neuroblastoma were irradiated with low doses X-ray as described above (Lambin et al., 1996). The cell line Be 11 was considered as radio-resistant - the SF 2 Gy ranged from 60 to 70 % - but the cell lines MeWo, SW 48 and HX 142 were radio-sensitive tumors with an SF 2 Gy ranging from 3 to 29 % (Lambin et al., 1996). The response obtained for doses ranging from 2 – 5 Gy for all cell lines fit with the LQ model, but HRS at doses < 0.5 Gy was not observed for the cell lines MeWo, SW 48 and HX 142 (Lambin et al., 1996). This absence of HRS in radiosensitive cell lines could be explained by the decreased inducible response of these cell lines (Lambin et al., 1996).

Human glioblastoma is considered to be one of the most radio-resistant tumors. Short et al studied five human glioblastoma cell lines, T98G – A7 – U87MG – U138 – HGL21, and one cell line derived from an anaplastic astrocytoma, U373 (Short et al., 1999a, 1999b). All the cell lines were irradiated with low doses of X-ray (Short et al., 1999a, 1999b). Survival time was calculated for the T98G – A7 – U373T9 lines using the DMIPS method. Survival time for U87MG – U138 – HGL21 which are not suitable for the DMIPS methods, were obtained from the cell shorter (CS) protocol as modified by Wouters et al (Wouters et al., 1996). HRS was noted at very low doses of X-rays in all five of the human glioblastoma cell lines, and most markedly in the A7 – U138 – TG98 cell lines (Short et al., 1999a, 1999b). The grade III cell line, U373, did not express HRS, though no clear explanation for this was put forward; possibly limitations of the CS methods or because this cell line could expresses HRS at much lower doses than is technically possible to test (Short et al., 1999a, 1999b).

To date, the low-dose responses have been reported by several laboratories in more than 26 different human cell lines, and survival times obtained using both DMIPS and the colony assay formation (CFA) (Beauchesne et al., 2003; Joiner et al., 1986, 1993a, 1993b; Lambin et al., 1993a, 1993b, 1994a, 1994b, 1994c, 1996; Marples et al., 1994, 1997; Short et al., 1999a, 1999b, 2001; Singh et al., 1994; Turesson & Joiner, 1996; Wouters et al., 1994, 1996). These include cell lines from colorectal carcinoma, bladder carcinoma, melanoma, prostate carcinoma, cervical squamous carcinoma, lung adenocarcinoma, neuroblastoma, gliomas, one non-malignant lung epithelial line, and one primary human fibroblast line.

Beauchesne et al also studied the HRS phenomenon in a French laboratory, using a linear accelerator to deliver the daily the radiation therapy for hospitalized patients (Beauchesne et al., 2003; Pedeux et al., 2003). The following human malignant cell lines established in this laboratory and previously described were tested; G5 – CL35 (a clone derived from G5) – G111 – G142 – G152. Cell survival was calculated from the CFA technique. Three hours after plating, cells were exposed to X-rays delivered by a linear accelerator (X photons of 10 MeV, dose rate of 2.43 Gy/min) with the irradiator placed at a distance of 1 m from the target, and an irradiation field of 40 X 40 cm (Beauchesne et al., 2003; Pedeux et al., 2003). The irradiation doses ranged from 0.2 to 2 Gy. HRS was once more reported at doses lower than 1 Gy for the glioma cell lines G5-G111-G142-G152, though, CL35, a regular sub-clone of G5, failed to express this HRS phenomenon (Fig. 2) (Beauchesne et al., 2003; Pedeux et al., 2003).

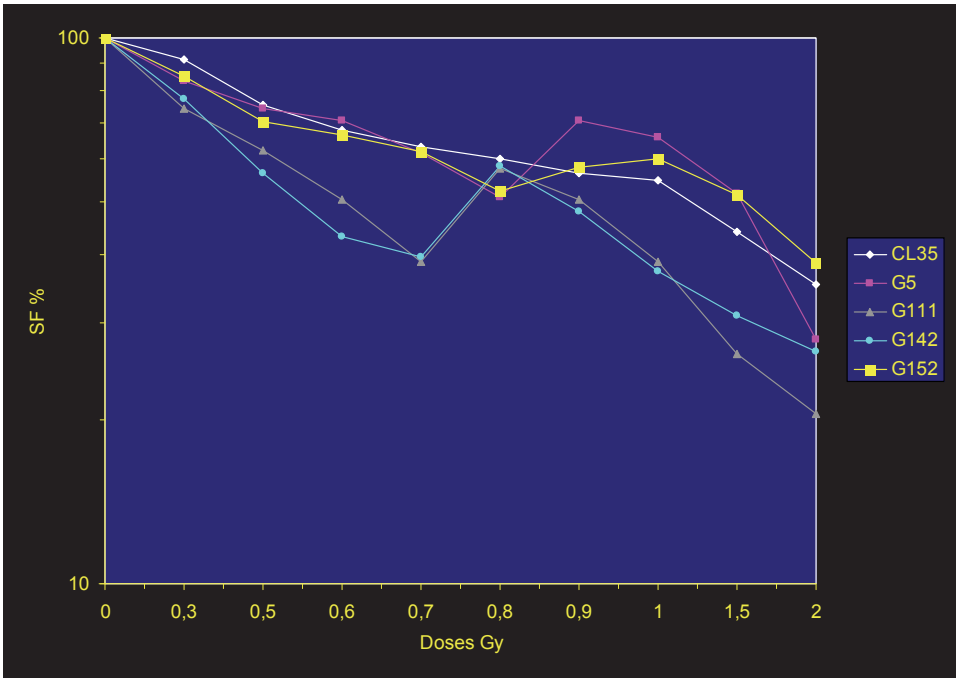


Fig. 2. Survival of human glioma cells following irradiation. Cells were irradiated with 0–2 Gy. G111, G142 and G152 glioma cell lines display HRS at doses below 1 Gy. HRS was observed only with G5 cells, whereas its clone, CL35, displayed conventional sensitivity to radiation therapy.

The authors demonstrated HRS in four human melanoma tumor cell lines, M4Be – A375P – MeWo – SKMe12, at doses below 1 Gy, and in the MRC5 human fibroblast cell strain (Beauchesne et al., 2003; Pedeux et al., 2003). HRS was not expressed in two radio-sensitive cell lines, H460 (from a lung cancer) and MCF7 (from breast cancer) (Beauchesne et al., 2003; Pedeux et al., 2003).
The same team (Beauchesne et al) also tested the chemotherapy combination etoposide – temozolomide concomitantly with low-doses fractions on the human glioblastoma cell lines, G5 – G142 – G152 (Beauchesne et al., 2003; Pedeux et al., 2003). Cells were incubated immediately after an ultrafractionated irradiation regimen with etoposide and temozolomide at determined doses for 24 hours. A marked radio-sensizitation effect was observed with the CL35 line, and an enhancement of the HRS phenomenon was reported for G142 and G152 (Beauchesne et al., 2003; Pedeux et al., 2003). Thus, the combination of chemotherapy and radiotherapy enhances the effects of the therapies, thus further improving the effect of repeated low-radiation doses on malignant glioma cells (Beauchesne et al., 2003; Pedeux et al., 2003).

2.2 Repeated irradiations

Short et al in another set of experiments using the T98G human cell line (derived from a human glioblastoma), tested low-doses irradiations given at < 0.5 Gy once or more daily

(Short et al., 1994a, 2001). Cell survival was calculated by DMIPS cell assay after 15 fractions of 0.4 Gy, given three times a day and compared to the same total dose given as once-daily 1.2 Gy. The low-doses were administered at 4-hours intervals (09.00 – 13.00 – 17.00 hours) each day for 5 consecutive days, and the single dose of 1.2 Gy was also given for 5 consecutive days (Short et al., 1994a, 2001). The repeated low-doses produced a significantly increased tumor cell kill; cell survival after three consecutive 0.4 Gy fractions was lower than after the same total dose given as a single fraction (1.2 Gy), the difference was significant ($p<0.0002$) (Short et al., 1994a, 2001). Cell survival after 2 Gy single doses was not different to that obtained after three consecutive 0.4 Gy fractions (Short et al., 1994a, 2001).Two other human glioblastoma cell lines, A7 – U87, were also tested: the lowest cell survival occurred with doses administered at 4 and 6 hours intervals for A7 and at 1 and 5 hours intervals for U87 (Short et al., 1994a, 2001). The cell line U373 (obtained from a human astrocytoma grade III) did not express HRS phenomenon, repeated low-doses did not enhance cell killing (Short et al., 1994a, 2001). The conclusions of this work were that multiple low-doses (< 1 Gy) per day spaced at appropriate intervals (4 hours) could increase cell killing by the enhancing he HRS phenomenon (Short et al., 1994a, 2001). The authors termed this multiple low-doses per fraction per day as an "ultrafractionated regimen" (Short et al., 1994a, 2001).

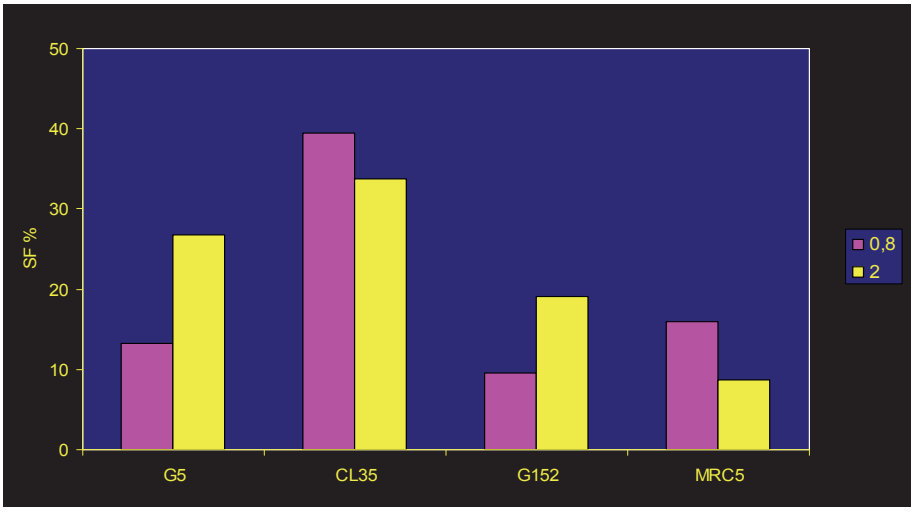


Fig. 3. Survival of human glioma cells following repeated irradiations. G5, CL35, G152 or MRC5 cells were exposed to 0.8 Gy 3 times/day spaced by 4 hr for 2 consecutive days or to 2 Gy once/day for 2 consecutive days. Cell survival was assessed by a clonogenic assay.

Beauchesne et al also tested the cumulative effect of low radiation doses on cell survival on the following human glioblastoma cell lines; G5 – CL35 – G152 (Beauchesne et al., 2003; Pedeux et al., 2003). Three fractions of 0.8 Gy spaced at 4 hour intervals were compared to a biologically equivalent single dose of 2 Gy. Irradiations were given for 2 consecutive days. A marked increase in cell killing was reported with the ultrafractionated regimen (repeated low-doses) in the G5 and G152 cell lines, but not in the CL35 cell line (Fig. 3) (Beauchesne et al., 2003; Pedeux et al., 2003). The experiments were repeated with a linear accelerator used daily for clinical therapies for patients. G5 and CL35 cell lines were exposed to 0.8 Gy, three times per day, spaced at 4 hour intervals for 2 consecutive days and to 2.4 Gy once a day for

2 days. Again, a marked and significant increase in cell killing occurred after the repeated low-doses for G5 but not the CL35 cell line (Beauchesne et al., 2003; Pedeux et al., 2003). It was postulated that te HRS phenomenon was responsible for the lower cell survival obtained after ultrafractionated regimen (Beauchesne et al., 2003; Pedeux et al., 2003).

3. *In Vivo* experiments

The first study which tested ultrafractionated irradiation on an animal model was reported by Beck-Bornholdt; the rat rhabdomyosarcoma R1H was irradiated with 126 fractions over 6 weeks. Top-up irradiations were not given (different doses per fraction between 0.43 and 0.71 Gy were applied) (Beck-Bornholdt et al., 1989). The results were compared to "historical control", and the authors demonstrated that the ultrafractionated regimen was slight more effective than the conventional approach (Beck-Bornholdt et al., 1989).

With a view to demonstrating a potential therapeutic benefit of the ultrafractionated irradiation schedule for malignant glioma patients, Beauchesne et al tested the fractionated low-dose irradiation in a glioma animal model, previously developed by the same team, on the G152 cell line (Beauchesne et al., 2003; Pedeux et al., 2003). Briefly the model was developed as follows; G152 malignant glioma cells (2×10^6) suspended in 0.1 ml of PBS were subcutaneously injected into the inter-scapular region of 4-week-old mice (female nude mice, Swiss *nu/nu*). Drinking water was supplemented with estrone (0.1 ml/100 ml of water) until death of the animal. Two perpendicular diameters (D1 and D2) of the tumors were measured once a week and tumor volume calculated from the following equation: $(D1 + D2/2)^3 \times (\pi/6)$ (Beauchesne et al., 2003; Pedeux et al., 2003). G152 xenograft tumors were grown for 17 days, and the mice were then exposed to either 0.8 Gy per fraction (3 times per day, spaced at 4 hour intervals, 4 days per week, for 2 consecutive weeks) or to a single dose of 2 Gy (once per day, 4 days per week, for 2 consecutive weeks). Another arm of tumor-bearing mice were not treated. The ultrafractionated irradiation was delivered by a clinical linear accelerator, with the mice immobilized in plastic tubes, and only the tumor exposed to the irradiation (Beauchesne et al., 2003; Pedeux et al., 2003).

Tumors grew faster in the untreated mice with an average tumor volume at week 12 of 1223 mm^3. As expected, radiation therapy had a therapeutic effect on the tumor growth resulting in an inhibition of tumor growth of 80-90 % (Beauchesne et al., 2003; Pedeux et al., 2003). At week 12, tumor volume of the mice in the ultrafractionated arm (repeated low-doses) was half that of the mice in the standard treatment arm (single dose, each day) representing a highly significant difference ($p=0.0022$) (Beauchesne et al., 2003; Pedeux et al., 2003). A second experiment gave similar results with neuropathology analysis revealed that the grafted tumor had the same characteristics as the initial human primary glioma tumor from which the G152 was obtained.

To further assert the therapeutic efficiency of ultrafractionated regimen, a third experiment was performed to compare the irradiation regimens for the same total doses (Beauchesne et al., 2003; Pedeux et al., 2003). Seventeen days after grafting, the mice were exposed to either 0.8 Gy, 3 times/day spaced at 4 hour intervals 5 days/week for 2 consecutive weeks (total dose = 24 Gy) or to 2.4 Gy once/day 5 days/week for 2 consecutive weeks (total dose = 24 Gy). Another group of mice was left untreated. Tumor size was measured once a week. As previously demonstrated, the ultrafractionated regimen led to a dramatic inhibition of tumor growth, and in the group of mice irradiated with fractions of 2.4 Gy, the tumor growth was not very different from mice irradiated with 2 Gy per fractions (Fig. 4)

(Beauchesne et al., 2003; Pedeux et al., 2003). These experiments show that ultrafractionated irradiation provides a marked benefit compared to a more classical irradiation regimen. It is worth noting that the use of a clinical linear accelerator is more feasible and the ultrafractionated regimen could thus be suitable for clinical treatment (Beauchesne et al., 2003; Pedeux et al., 2003).

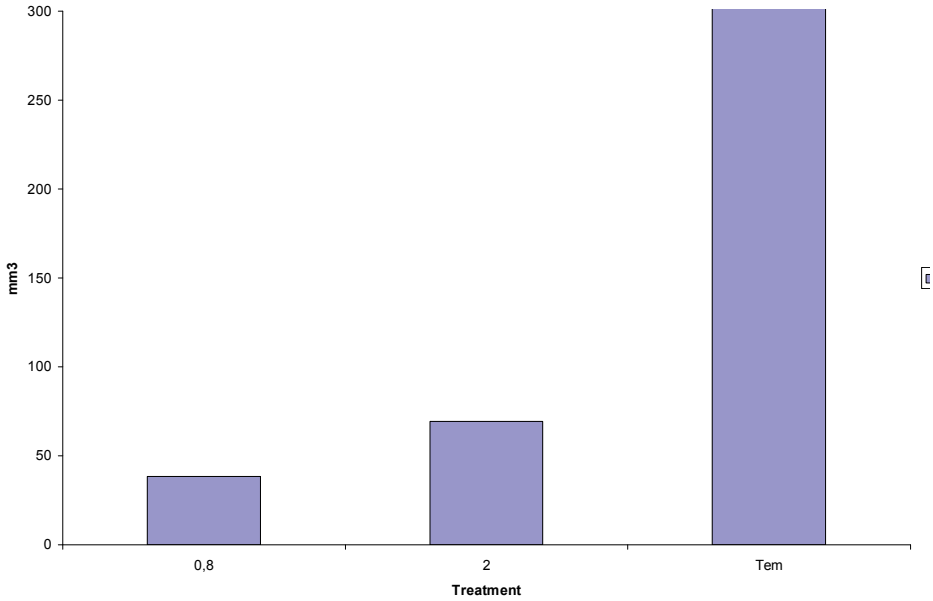


Fig. 4. Inhibition of glioma tumor growth following repeated irradiation with low doses. G152 glioma cells injected into the interscapular region of 4-week-old female nude mice (Swiss *nu/nu*). Seventeen days after grafting, mice were exposed either to 0.8 Gy 3 times/day spaced by 4 hr 5 days/week for 2 consecutive weeks or to 2.4 Gy once/day 5 days/week for 2 consecutive weeks. Tumor size was measured once a week.

Krause et al tested the ultrafractionated regimen in an animal model grafted with cells derived from the A7 human cell line which expressed the HRS phenomenon (Krause et al., 2003). Cryoprereserved tumor tissue was transplanted subcutaneously onto the backs of five mice. The tumor with the median volume doubling time was transplanted onto the back of another eight to 12 mice. 1–2 mm pieces of tissue from the median tumor were then transplanted subcutaneously into the right hind leg of the experimental animals (Krause et al., 2003). Irradiation protocols were started daily when the tumours reached a mean diameter of 5 mm, corresponding to a volume of 57 mm^3; the ultrafractionated regimen consisted of 26 fractions over 6 weeks (0.4 Gy per fraction, 3 fractions per day, 21 fractions per week, spaced at 6 hour intervals), and conventional treatment consisted of 30 fractions over 6 weeks (1.68 Gy per fraction, once daily, 5 fractions per week) (Krause et al., 2003). A local irradiator was used. Endpoints were tumor growth delay and local tumor control 180 days after the end of the treatment, and for the first 60 days after the end of irradiation. The tumors were measured twice weekly and once weekly thereafter (Krause et al., 2003). The

authors were unable to demonstrate a therapeutic benefit of the ultrafractionated regimen; growth delay in the ultrafractionated arm was significantly shorter than after conventional treatment (Krause et al., 2003).The top-up TCD_{50} values were 28.3 Gy for conventional irradiation and 40 Gy for the ultrafractionated regimen (p=0.047) (Krause et al., 2003). The use of a local irradiator with a dose rate 0.2 – 0.4 Gy/min could alter the molecular mechanisms and perhaps modify the mechanisms responsible for the HRS phenomenon (Krause et al., 2003).

4. Clinical trials

The ultrafractionated radiation therapy regimen

To translate these *in vitro* and *in vivo* observations into a clinical setting, Beauchesne et al initiated a phase I/II clinical trial using an ultrafractionated radiation therapy protocol (3 times 0.75 Gy for a total of 67.5 Gy) (Beauchesne et al., 2010). The main purpose of this study was to assess the toxicity of the ultrafractionated regimen. This protocol was initiated before concomitant radio-chemotherapy became standard of care. For this pilot trial, the authors purposely selected patients with unfavourable clinical prognostic factors: newly unresectable glioblastoma (Beauchesne et al., 2010).

4.1 The population

This phase I/II study was conducted in seven French centres. Patients (> 18 years and who were able to give informed consent) with newly diagnosed, supratentorial, unresectable but histologically confirmed glioblastoma (astrocytoma grade IV according to the WHO classification), with a WHO performance status of 0-2 were eligible (Beauchesne et al., 2010). Patients were included based on local pathology. Patients with an estimated survival of less than 3 months, who had undergone partial or complete tumor resection, or who had previously received prior radiation therapy were not eligible. The primary end-points of the study were the treatment-related toxicity and tolerability (Beauchesne et al., 2010). Secondary end-points were progression-free survival (PFS) and overall survival (OS) of glioblastoma patients (Beauchesne et al., 2010).

4.2 Treatment

The radiation therapy regimen consisted of ultrafractionated focal irradiation with three daily doses of 0.75 Gy delivered at least four hours apart five days a week (Monday through Friday), for 6-7 consecutive weeks. A total dose of 67.5 Gy was delivered in 90 fractions (Beauchesne et al., 2010). Irradiation was delivered to the gross tumor volume with a 2.5 cm margin for the clinical target volume. Radiation therapy was planned with dedicated computed tomography (CT) or magnetic resonance imaging (MRI), and three-dimensional planning systems; conformal ultrafractionated radiotherapy was delivered with linear accelerators with a nominal energy of 6 MeV or more (Beauchesne et al., 2010). The patients were treated with thermoplastic immobilization masks to ensure adequate immobilization and reproducibility.

4.3 Patient evaluation

The patients were assessed weekly for tolerability and toxicity during the radiation therapy. The baseline examination included cranial MRI (with and without contrast), physical and

neurological examination and included Mini-Mental-Status score (MMS) and a quality-of-life questionnaire (EORTC – QLQ-C30, Brain Cancer Module BN-20) (Beauchesne et al., 2010). Baseline examination was performed at the end of radiation therapy regimen (within the first 10 days after completion of ultrafractionated irradiation) and then every 2 months until death. The first MRI (at the end of radiation therapy) constituted the baseline imaging to evaluate tumor response keeping in mind that radiation therapy artifacts if present should be taken into account when interpreting the images (Beauchesne et al., 2010). Tumor progression was defined according to the modified WHO criteria (Macdonald criteria) as an increase in tumor size by 25 percent (size of the product of the largest perpendicular diameters of contrast-enhancing tumor), the appearance of new lesions, or an increased need for corticosteroids (MacDonald et al., 1990). When there was tumor progression, patients were treated at the investigator's discretion, and the type of subsequent therapy (usually chemotherapy) was recorded (Beauchesne et al., 2010).

4.4 Patient characteristics

From September 2003 until June 2006, 31 patients were enrolled in this phase II study (16 males and 15 females). Median patient was 58 years (range 37 to 76). Median Karnofsky Performance Status (KPS) was 80, ranging from 60 to 100 (Beauchesne et al., 2010). The median time from diagnosis to the beginning of ultrafractionated radiation therapy was 6 weeks (ranging from 2 to 10 weeks) (Beauchesne et al., 2010). Four patients died before the beginning of irradiation and two decided to revert to the standard radiation therapy regimen after starting (Beauchesne et al., 2010). The radiation course was completed in 22 patients. Multi-focal glioblastoma was diagnosed in seven patients, four of whom received and completed the ultrafractionated regimen (Beauchesne et al., 2010). Neuropathology was review reviewed by a central laboratory and all but one case was diagnosed as glioblastoma as WHO classification, and one case was secondary and classified as anaplastic oligodendroglioma.

4.5 Safety and tolerability

No toxic death occurred during the ultrafactionated irradiation and no radiation therapy regimen was discontinued. All but three patients (25 patients) received the ultrafractionated irradiation, and 22 completed the course of the treatment (Beauchesne et al., 2010). Two patients with a very large tumor progressed during the radiation therapy, and radiation therapy leading to premature discontinuation after 48 and 56 Gy. The most common adverse event was fatigue, as is frequently observed in standard cranial radiation therapy (Beauchesne et al., 2010). Although the ultrafractionation regimen was a constraint to patients, it was well accepted; only one out of 25 changed his mind during the course of the treatment, and was withdrawn. Overall, the ultrafractionated regimen was well tolerated.

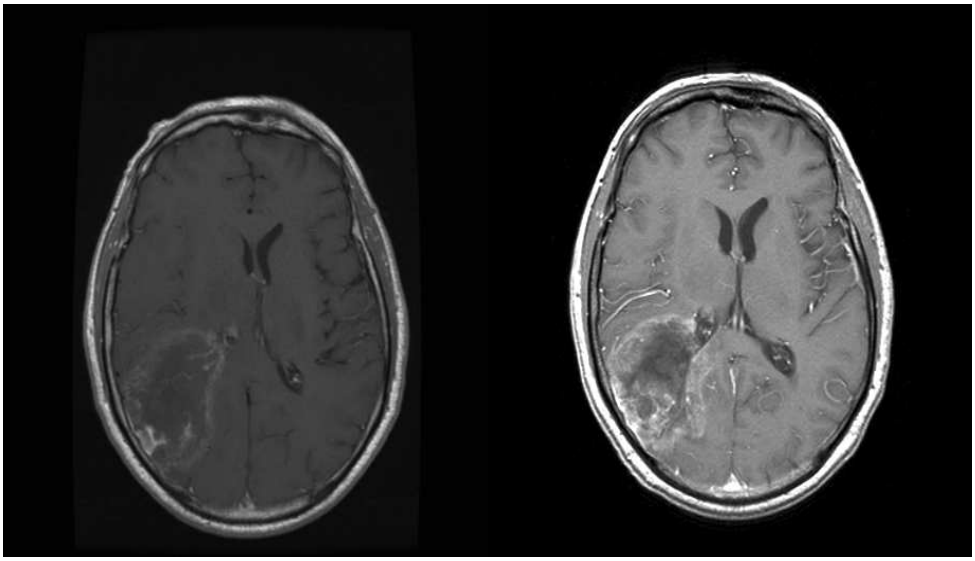
4.6 Survival

After a median follow-up of 4 years, two of the 31 initial patients were alive (6.5 %). The median survival was 9.53 months (Beauchesne et al., 2010). The OS at 6, 12, 18 and 24 months was respectively 74.19 %, 29.03 %, 19.35 % and 15.48 %. The median PFS was 5.09 months. The PFS at 6, 12, 18, and 24 months was respectively 45.16 %, 12.90 %, 6.45 % and 6.45 % (Beauchesne et al., 2010). No difference was found in the median survival for age and sex: 8.4 months for males vs. 8.9 months for females, 8.4 months for < 55 years vs. 9.5

months for > 55 years (Beauchesne et al., 2010). The trial design allowed the use of fotemustine, a nitrosourea component, as first line of chemotherapy at tumor progression, but patients were treated at the physician's discretion. At the cut-off date, 16 patients had received chemotherapy; fotemustine was given in 14 cases, and temozolomide in 2 (Beauchesne et al., 2010). One patient underwent surgery, and radio-surgery was performed in one case. The response to salvage chemotherapy was not recorded as part of our study.

4.7 Response to treatment

Tumor response was analyzed (at the end of the ultrafractionated regimen and then 2 months later) using the WHO criteria. Eight stabilizations (corresponding to a decrease of less than 25 % of perpendicular transversal diameters – no cerebral edema and mass effect) were observed among the patients who had received the ultrafractionated radiation therapy (Fig. 5) (Beauchesne et al., 2010). In addition, the doses of corticosteroids were stable or decreased for these patients. Stabilization of tumor responses was observed either at the end of radiation therapy or in the following months. In the remaining cases, the tumor was non-progressive (Beauchesne et al., 2010). In most cases, corticosteroids were decreased and stopped for several weeks. Half of the long term survivors did not receive a chemotherapy line.



A MRI scan B MRI scan.

Fig. 5. A stabilisation response was seen on a cranial MRI. A- At the beginning of treatment. B- At the end of radiotherapy.

4.8 Commentaries

Before this study was conducted, the safety and tolerability of this new regimen of radiation therapy, three doses per day administered at intervals at least 4 hours, were unknown. This was the first time that such an ultrafractionated radiation therapy regimen was clinically

performed (Beauchesne et al., 2010). The study showed that the regimen is safe, well tolerated and well accepted by the patients. No toxic death occurred and no neurological symptoms evoking a post-radiation therapy leuco-encephalopathy were recorded (Beauchesne et al., 2010). Neither were any abnormal radiological findings suggesting a potential toxic effect of ultrafractionated irradiation noted on cranial MRI. Fatigue, as is usually observed with the standard cranial irradiation, was the main adverse event recorded.

The median survival compares favorably with the best outcomes observed in the recent large randomized studies (Athanassiou et al., 2005; Westphal et al., 2003). Moreover, these studies had included a high percentage of patients with better prognosis (good WHO performance status – young age – resection of tumor – a single tumor), and the adjuvant chemotherapy was systematically administered (Athanassiou et al., 2005; Westphal et al., 2003). The EORTC-NCIC study reported a median survival of 7.85 and 9.4 months respectively for radiation therapy alone and temozolomide/radiotherapy together administered to patients with inoperable glioblastomas (Stupp et al., 2005).

Beauchesne et al's study resulted in both a longer median survival time and higher OS at 24 months (9.53 months – 15.38 % and 7.4 months – 8 % respectively) than the RTOG 90-06 trial (7.4 months and 8 % respectively) (Beauchesne et al., 2010; Scott et al., 1998). Furthermore, there were an unexpectedly high number of long survivors; 19.35 % and 15.48 % at 18 and 24 months. This compares favorably with the 24 months survival in the EORTC-NCIC study which stands at 4.59 % for the radiation therapy alone arm and 10.42 % for the temozolomide/radiation therapy arm (Beauchesne et al., 2010; Stupp et al., 2005). Furthermore half of the patients in the Beauchesne et al trial did not receive a chemotherapy line or other therapy (Beauchesne et al., 2010). It seems reasonable therefore to claim an ultrafractionated radiation therapy regimen may result in better long survival and OS compared with the best results reported in the literature.

These encouraging results support the development of a randomized phase II study to test the efficacy of a concomitant combination of ultrafractionated radiation therapy and temozolomide in no-operable glioblastomas. Beauchesne et al initiated such a study in February 2008 in France to test this new combination of ultrafractionated irradiation and temozolomide. Patients over 18 years of age who are able to give informed consent and have histologically proven, newly diagnosed inoperable and supratentorial glioblastoma are eligible. Three doses of 0.75 Gy are delivered daily at a minimum of 4 hour intervals, 5 days a week for 6 consecutive weeks (67.5Gy). Concomitant chemotherapy consisting of temozolomide is given 7 days per week during the ultrafractionated radiation therapy. After a 4-week break, chemotherapy is resumed with up to 6 cycles of adjuvant temozolomide every 28 days. Tolerability and toxicity are the primary endpoints and survival and PFS the secondary endpoints.

To date 36 patients have been enrolled in this study, 24 men and 12 women with a median age of 62, years and median KPS of 80. The ultrafractionated radiation therapy - temozolomide combination has been well tolerated; no acute grade 3 and/or 4 CNS toxicity has been observed and only one grade 4 hematological toxicity has been reported. Two patients progressed during the radiation therapy, and two patients died of pulmonary embolism. Median survival has not yet been reached. Half of the patients have survived for more than one year.

5. Conclusions

The "Low Dose Hypersensitivity" phenomenon has been demonstrated *in vitro* in a number of various human malignant glioma cell lines, when cells were irradiated with single-doses of X-rays from 0.05 to 5 Gy, and focusing on cell survival at doses less than 1 Gy. Interestingly, daily repeated irradiation of cells with low doses compared to irradiation with a single biologically equivalent dose resulted in significantly higher cell kill (as measured with a clonogenic assay).

Experiments conducted on glioma xenografts went on to demonstrate that repeated irradiation with low doses (0.8 Gy, 3 times a day) is more effective than a single dose (2 or 2.4 Gy, once a day) to inhibit tumor growth.

Clinical trials on ultrafractionated radiation regimens confirm these experimental results and have proved to be safe and well tolerated. No acute grade 3 and/or 4 CNS toxicity has been observed in such trials. Median progression-free and survival from initial diagnosis have been found to be 5.1 and 9.5 months. When compared with the EORTC/NCIC trial in both PFS and OS multivariate analysis, ultrafractionation showed superiority over radiation therapy alone but not over radiation therapy and temozolomide. Nevertheless, the treatment regimen has proved feasible, well tolerated and deserves to be further evaluated in combination with the current standard concomitant chemotherapy agents.

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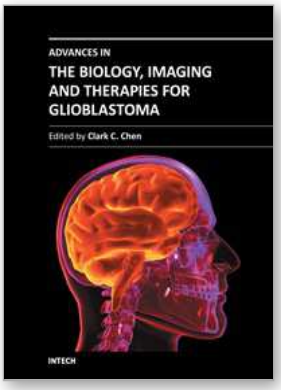

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This book is intended for physicians and scientists with interest in glioblastoma biology, imaging and therapy. Select topics in DNA repair are presented here to demonstrate novel paradigms as they relate to therapeutic strategies. The book should serve as a supplementary text in courses and seminars as well as a general reference.

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