

Title

Safety of intensity-modulated radiotherapy treatment with inhomogeneous dose distribution in patients with relapsed high-grade gliomas

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KEYWORDS

Relapsed glioblastoma

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Dose painting

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SYNOPSIS

Title of the study	<ul style="list-style-type: none"> • Safety of intensity-modulated radiotherapy treatment with inhomogeneous dose distribution in patients with relapsed high-grade gliomas
Study design	<ul style="list-style-type: none"> • Exploratory, prospective, experimental and monocentric study.
Primary goal	<ul style="list-style-type: none"> • Assessment of acute and subacute toxicity of radiotherapy treatment.
Secondary objectives	<ul style="list-style-type: none"> • Evaluation of the response rate according to the RANO criteria: local control of the disease. • Incidence of treatment-related radionecrosis. • Evaluation (descriptive) of the Quality of Life assessed through EORTC QLQ-C30 questionnaires. • Evaluation (descriptive) of neuro cognitive functions as indicated below. • Overall survival (OS) assessment. • Assessment of progression-free survival (PFS).
Inclusion criteria	<ul style="list-style-type: none"> • GBM relapsed after standard surgery-radio-chemotherapy treatment or other therapeutic lines, or GBM secondary to anaplastic astrocytoma previously treated with radio and chemotherapy, or relapses of anaplastic astrocytoma with clear radiological signs of change. • Age \geq 18 years.

	<ul style="list-style-type: none"> • ECOG Performance Status 0-2, Performance Karnofsky Score > 60. • Written informed consent. • Life expectancy > 3 months. • Availability of the patient to be followed for all phases of chemotherapy treatment and for the subsequent follow-up.
Exclusion criteria	<ul style="list-style-type: none"> • Patients with KPS < 70%. • Participation in other studies that involve the administration of experimental drugs or explicitly exclude the possibility of participating in other studies in general or in studies whose characteristics include aspects of this study. • Any concomitant medical or psychological conditions that could prevent participation in the study or that compromise the ability to provide informed consent. • Pregnant and breast-feeding patients.
Treatment	<ul style="list-style-type: none"> • The treatment provided for in the protocol involves the planning and execution of a hypofractionated radiotherapy treatment, administered in 5 daily sessions, with modulated intensity technique and uneven distribution of the dose guided by diffusion magnetic resonance images (MRI).
Sample of the study	<ul style="list-style-type: none"> • 12 patients
Expected date of start and end of the study	<ul style="list-style-type: none"> • 25 months: 22 months of enrolment + 3 months of follow-up

1. INTRODUCTION

The standard therapy for glioblastoma multiforme (GBM) patients has been surgery and radiotherapy for many years. In 2005, the results of a major multicenter Phase III study conducted by the EORTC and the NCIC were published which demonstrated that the addition of Temozolomide chemotherapy to surgery and postoperative radiotherapy can lead to an increase in overall (OS) and progression-free (PFS) [1] survival. However, the prognosis of these patients remains very severe: the recent update of the EORTC / NCIC trial data demonstrated a median survival of 14.6 months (range, 15-18 months) [2], with a local relapse rate of over 90%. Less than 10% of patients are alive at 5 years [3]. More than 90% of all GBMs recur in the vicinity of the surgical cavity and in any case within the radiotherapy volume [4].

To date, many salvage therapeutic approaches are available for relapsed GBMs after surgery-radio-chemotherapy (Stupp protocol), such as re-intervention, chemotherapy or re-irradiation, although an optimal standard does not yet exist. Systemic therapy is the most frequent option for GBM relapses, but the literature data report an overall survival with chemotherapy alone of 4-6 months from relapse [5]. Recent series report encouraging results with TMZ monotherapy with traditional or intensification schedules (dose-dense schedules) with a PFS of 30% [6,7,8,9].

Unfortunately, considering the small number of patients enrolled in these studies and the large variability of therapeutic regimens, there is no evidence today that one regimen is more beneficial than another, and despite an increase in PFS, there does not appear to be any increase in OS with none of the systemic approaches tested. Bevacizumab was recently approved by the US Food and Drug Administration for the treatment of relapsed GBM with phase II studies that reported an increase in PFS and OS at 6 months [10].

In the context of relapsed GBMs, re-irradiation has always been a controversial topic due to the risk of toxicity. The tolerance of healthy brain tissue to possible re-irradiation depends on many factors, such as the daily dose, the total dose, the total treatment time, the interval since the previous radiotherapy, the volume of healthy tissue already irradiated, the additional therapies and many other factors [11, 12]. But recently some studies have been published which, in the face of limited radio-induced toxicity, have reported good results in terms of local control with re-irradiation of relapsed GBMs [13-15]. Furthermore, the technological innovation of recent years in the field of radiotherapy, with the advent of more sophisticated delivery techniques such as intensity modulation radiotherapy (IMRT) or stereotaxic radiotherapy (SRT), allows us today to affirm that re-irradiation of malignant brain neoplasms is effective and safe in terms of both acute and subacute toxicity. The most used radiotherapy approach is undoubtedly stereotaxic radiotherapy and many data are now present in the literature with a median survival of 6 to 10 months from the diagnosis of relapse, with low rates of toxicity [13-21]. Some more recent series have also evaluated the association of re-irradiation with systemic therapies, reporting a median OS of 8-13 months and a PFS of 5-8 months [22-26].

Table 1 summarizes all the studies published to date on the re-irradiation of GBMs with various techniques and radiation doses.

Table 1 – Clinical studies analysed

Re-irradiation with external beam radiotherapy with or without chemotherapy			
Authors	Patients (n)	Total dose/fractions	Outcomes from the re-irradiation
Bauman et al.	10	18-74 Gy 1,8-2 Gy x fraction	median OS 8.3 months median PFS 3.3 months
Kim et al.	14	36	median OS 7 months
Hayat et al.	11	30/2.5 Gy	median OS 13 months
Arcicasa et al.	24	34.5	median OS 13.7 months
Nieder et al.	31	45.4 bid	median OS 8.5 months median PFS 5 months
Verninga et al.	22	46	median OS 6.1 months GBM 8.2 months HGG
Henke et al.	21	20	median OS 10.2 months

Osman et al.	29 (20 GBM, 9 HGG)	30-40 Gy/ 1.8 Gy/ fraction	median OS 11 months median PFS 10 months
Re-irradiation with fractionated stereotactic radiotherapy with or without chemotherapy			
Cho et al.	20 (15 GBM)	Median dose of 37.5 Gy (range, 20-45 Gy) /2.5 Gy fractions (range, 1.8-3 Gy)	Median survival 12 months
Combs et al.	40	36 Gy/2 Gy fractions	Median OS 16 months Median PFS 8 months
Combs et al.	50	36 Gy/2 Gy fractions	Median OS 8 months Median PFS 5 months
Vordermark et al.	19 (14 GBM)	Median total dose 30 Gy (range, 20–30 Gy) /5 Gy fractions (range, 4–10 Gy)	Median OS 9.3 months Median PFS 4.9 months GBM 7.9 months HGG 15.4 months
Grosu et al.	44 (33 GBM)	30 Gy	Median OS 8 months
Laing et al.	19 (12 GBM)	40 Gy	Median OS 9.8 months
Selch et al.	21 (14 GBM)	25 Gy	Median OS 6.7 months Median PFS 4 months
Wurm et al.	25 (20 GBM)	25-30 Gy	Median OS 14.5 months Median PFS 10.5 months
Kolshi et al.	25 (11 GBM)	28.1-68.2 Gy	Median OS GBM 11 months Median OS HGG 19 months
Ciammella et al.	15	25 Gy/5 fx	Median OS 9.5 months
Ernst-Stecken et al.	15 (11 GBM)	35 Gy/7 Gy fractions	6 months PFS 75% 12 months PFS 53%
Fokas et al.	53	Median dose 54 Gy (range 20-60 Gy)/ 3 Gy fractions (range 2-5 Gy)	Median OS 9 months 1-year PFS 22% 2-year PFS 5%
Schwer et al.	15 (11 GBM)	18-36 Gy	Median OS 10 months Median PFS 7 months 6 months PFS 63%
Coombs et al.	25	36 Gy	Median OS 8 months Median PFS 5 months 6 months PFS 48%

Henke et al.	31 (2 grade III, 29 grade IV)	Median total dose 20Gy (range, 20–25)/ 5 Gy fractions	Median OS 10.2 months
Patel et al.	10	36 Gy	Median OS 7.4 months
Gutin et al.	25 (20 GBM)	30 Gy	Median OS 12.5 months Median PFS 7.5 months 6 months PFS 64%
Fogh et al.	147 (42 grade III, 105 grade IV)	Median dose 35 Gy in 3.5- Gy fractions	Median OS 11 months for grade III and 8 months for grade IV
Hauff et al.	59	30 Gy + thermotherapy	Median OS 13.4 months
Villaceincio et al.	26	20	Median OS 7 months
Torcuator et al.	16	36 Gy	Median OS 7.2 months Median PFS 2.6 months
Minniti et al.	36	37.5 Gy	Median OS 9.7 months Median PFS 3 months 6 months PFS 42%
Shepherd et al.	29	Median dose 35 Gy (range, 20–50 Gy)/ 5 Gy fractions	Median OS 10.7 months
Glass et al.	20 (7 grade III, 13 grade IV)	Median dose 38 Gy (range, 35–42 Gy)/ 3.5–6 Gy fractions	Median OS 12.7 months
Hudes et al.	20 (19 GBM)	Median dose 30 Gy (range, 24–35 Gy)/ 3–3.5 Gy fractions	Median OS 10.5 months
Lederman et al.	88	Total dose 18–36/ 4–9 Gy (weekly)	Median OS 7 months
Voynov et al.	10 (6 grade III, 4 grade IV)	30 Gy /5 Gy fractions	Median OS 10.1 months
Wuthrick E.J et al.	11	36 Gy (range, 30-42 Gy in 2.5 – to 3.75 Gy fractions)	6 months PFS 45%
Greenspoon et al.	32	25-30 Gy	Median OS 9 months Median PFS 7 months 6 months PFS 60%

Re-irradiation with radiosurgery			
Alexander et al.	25 (16 GBM)	13 Gy to 79% isodose	Median OS 9 months
Chamberlain et al.	13 (5 GBM)	Mean 12 Gy for GBM Mean 14 Gy for HGG	Median OS 8 months Median PFS 4 months
Shrieve et al.	86	13 Gy to med. 80% isodose	Median OS 10.2 months
Larson et al.	63 (46 GBM)	Med. Min. 16 Gy to med. 50% isodose	Median OS 14 months
Kondziolka et al.	42 (19 GBM)	Mean 15.5 Gy for GBM Mean 15.2 Gy for HGG to 50% isodose	Median OS 30 months for GBM Median OS 31 months for HGG
Cho et al.	46 (27 GBM)	17 Gy to med. 50% isodose	Median OS 11 months
Park et al.	23	15 Gy to 60% isodose	Median OS 10.3 months Median PFS 4.7 months
Larson et al.	26 (14 GBM)	Med. Min. 15 for GBM 16.5 Gy for HGG	For GBM: Median OS 9.5 months Median PFS 3.7 months For HGG: Median OS 17 months Median PFS 7 months
Combs et al.	32	15 Gy to 80% isodose	Median OS 10 months Median PFS 5 months
Hsieh et al.	26	12 Gy to 50% isodose	Median OS 10 months
Mahajan et al.	41	n.r.	Median OS 11 months
Kong et al.	114 (65 GBM)	16 Gy to 50% or 80% isodose	For GBM: Median OS 13 months Median PFS 4.6 months For HGG: median OS 26 months Median PFS 8.6 months
Biswas et al.	18	15 Gy to the isocenter	Median OS 5.3 months Median PFS 3.4 months
Patel et al.	26	18 Gy to 90% isodose	Median OS 8.4 months
Maranzano et al.	13	17 Gy to the isocenter	Median OS 11 months

Martínez-Carrillo et al.	87 (46 GBM, 41 AA)	18 Gy to the isocenter	Median OS 10 months 7.5 months for GBM 17 months for AA
Dodoo et al.	55 (35 GBM, 20 AA)	Median prescription dose 20 Gy (14-22 Gy)	Median OS 11.3 months for GBM Median OS 24.2 months for AA
Pouratian et al.	26	Median dose of 17 Gy to 70% isodose	Median OS 9.4 months
Cabrera et al.	15	24 or 18 Gy in a single fraction 25 Gy total in 5 consecutive daily 5- Gy fractions.	Median OS 14.4 months Median PFS 3.9 months 6-months PFS of 20%

Most of these studies are mono-institutional retrospective series conducted in past years with less sophisticated treatment delivery techniques than those available today, and above all with predominantly morphological imaging. Furthermore, many of these studies do not report how the site of recurrence was identified and with which imaging methods, but it is easy to assume that CT and NMR scans were used for the definition of gross tumor volume (GTV). On the latter, for years the only contrast point in T1-weighted sequences was identified as GTV without taking into account the surrounding edema and the functional characteristics of the area to be irradiated.

With modern high-field Magnetic Resonance (MRI) equipment, equipped with suitable hardware elements (high intensity gradients and broadband RF amplifiers), sophisticated in vivo analyzes and characterizations of the anatomical-functional structure of the human brain are possible. Advanced NMR techniques allow to perform measurements on microscopic scales by measuring effects not only ascribable in classical terms to direct measurements of relaxation times (T1, T2) and proton density, but also linked to more complex mechanisms of biochemical and biophysical cell interaction same.

In particular: the study of the energy distribution of nuclear spin levels, which makes it possible to measure the concentration and spatial distribution of metabolic complexes (Magnetic Resonance Spectroscopy, MRS); the study of the dynamics of the capillary flow which allows to obtain non-invasive tissue perfusion measurements (PWI) previously obtainable only with nuclear techniques (PET, SPECT); the study of the Brownian motion of water molecules in tissues through the estimation of the apparent diffusion coefficient (DWI) and / or the characterization of the Diffusion Tensor (DTI).

The introduction of dedicated contrast media, distinct in relation to their pharmacokinetics (interstitial contrast media and organ-specific contrast media) and their magnetic [paramagnetic (gadolinium chelates) and super-paramagnetic] characteristics, enhance the offered characteristics by the method and considerably increased its diagnostic power in clinical practice. The high levels

of spatial and temporal resolution that can be obtained and the absence of ionizing radiation also represent a further strength of the method.

These potentials are extremely effective in the study of brain tumors and in particular in the study of brain re-irradiations, where they can be applied in order to obtain a more correct characterization and definition of the target volume.

The integration between this advanced imaging modality and the new planning and delivery techniques of radiotherapy treatment can allow selective irradiation (dose painting) of pathological areas at different risk of progression through the identification of pathological sub-volumes. The new frontier of dispensing inhomogeneous dose within the target volume and adapting "the necessary dose levels" to the "true volume" is a more adequate approach in terms of tumor control probability than the more classic concept of distribution homogeneous dose. However, such a treatment modality requires sophisticated imaging sequences and new processing tools to be able to carry out an integrated and accurate evaluation of the pattern to be treated (creation of multi-parametric maps [79] that describe the different characteristics and / or properties of the tissues under examination), ad hoc software (see the Technical Annex) to create an appropriate segmentation of the tumor and an accurate dose planning, as well as a hi-tech linear accelerator (Varian TrueBeam STx), that allows a rapid delivery of the dose with submillimetric precision and continuous verification of the patient's correct set-up [75-78].

The segmentation of the different volumes to be treated is achieved by integrating the different NMR imaging sequences in multi-parametric maps, thanks to which it will be easier to proceed to a better definition of the GTV, CTV and PTV [80, 81]. The imaging that will be used will use conventional imaging sequences such as those weighed in T1 (T1w), T2 (T2w) and FLAIR, with (T1wGd) and without gadolinium contrast, but these data will be integrated with the information provided by the sequences more advanced than NMR: MRS [82], PWI [83] and DWI [84] or DTI [83, 84].

Brain spectroscopic imaging (MRS) provides information on neuronal integrity, cell proliferation and degradation, energy metabolism (concentration of metabolites), necrotic transformation of the brain or tumor tissue. Its use will provide valuable help in assessing the degree of cellular anaplasia of tumors, the anatomical extent of peri-tumor edema glioblastoma.

It will also represent a valuable tool in monitoring the evolution of neoplastic disease, especially after surgical and / or radiotherapy treatment, that is, in the differentiation of damage from radionecrosis - from residual or tumor recurrence.

Brain perfusion imaging (PWI) [84, 85], thanks to the close relationship between physiological function, metabolism and local blood supply, provides an in vivo measure of the regional cerebral hemodynamic of the area under examination. To evaluate the flow, the response of the system to an intravascular tracer is recorded over time, which can be both exogenous (gadolinium chelates) and endogenous (H₂O molecules present in the blood). Limiting ourselves to the first case (DSC-MRI technique), by applying mathematical models on the properties of blood transport and on the mechanisms of exchange with the tissue, we go back to the measurement of hemodynamic

parameters, such as cerebral blood flow (CBF), blood volume (CBV) and the Average Transit Time (MTT). The method provides information on the identification of areas of high angiogenesis and permeability, allowing to follow the evolution of the tumor residue and / or to discriminate between the areas of recurrence and / or radionecrosis, as well as being linked, albeit with a certain approximation, to the presence of oxygen in a tissue and therefore to its level of toxicity or hypoxia.

Diffusion Weighted Imaging (DWI) [84] provides a measure of water mobility which is a valid tool for describing tissue structure at microscopic scales, as water diffusion plays a fundamental role in transport processes enzymes, metabolic substrates and metabolites. Human tissues can only survive within a narrow temperature range in which most of the tissue components are in a liquid state and exhibit a highly inhomogeneous structure at the microscopic level (cell membranes and various organelles hinder the free movement of water and of other molecules). Neoplasm, characterized by uncontrolled cell reproduction, is characterized by an increase in tissue cellularity (cell density) in which the extra-cellular volume fraction (ECVF) decreases compared to the equivalent healthy tissue [86].

The signal intensity variation map can provide useful information such as mean diffusivity and diffusion anisotropy index (DTI), computational tracing of white matter fiber bundles (Fiber Tracking) or apparent diffusion coefficient (ADC) [86].

Thanks to the estimation of the ADC of the molecules it is possible to study the conformation of the tissues, their integrity and to classify the normal and pathological tissue using the respective ADC values: the high cellularity of the tumors determines a restriction of the diffusivity that produces low ADC values conversely, necrosis, which consists in a rupture of the membranes with cell death, causing an increase in the diffusivity of the water generates high ADC values [86, 87]. Although the complex interactions between water and tissue microstructures have not yet been fully understood, the scientific literature highlights the existence of an inverse proportionality between the cellularity of tissues and the ADC value [86 - 90]. This type of correlation makes it possible to propose, after having 'correctly' segmented the different areas of the tumor, the use of ADC values as a guide for dose redistribution (dose painting) with photon beams. Considering these values as a surrogate of cellularity, it is reasonable to hypothesize that low ADC values are correlated to high values of cell density and therefore to a high number of clonogens (which are a small fraction, $\leq 1\%$, of the number of cells that make up the whole neoplastic tissue) [91].

The higher dose values would therefore be focused on these values, while the higher ADC values would correspond to the lower dose values.

Recently the literature has shown that diffusion maps also seem to provide an early assessment of the tumor response to treatment [86 - 90], showing a variation in their distribution with respect to the response to treatment. Similar data were also found in the ADC pattern of some patients irradiated by us with conventional treatment (STUPP) and for which the trend of the parameters of such distribution was retrospectively monitored using indicators calculated with the Texture Analysis (TA). The TA [92, 93] is an analysis method that allows to study and evaluate the

distribution of the pixels (the punctual signal of each image) that make up the images, in our case the values of the NMR images, expressed in the form of indicators that describe the shape and the trend: mean, standard deviation, entropy, kurtosis, etc. The post-therapy reproduction of the pre-treatment ADC values, relative to the regions where the relapse has re-manifested and / or has not succeeded to control the neoplasm, is indicative of a non-response to treatment and therefore seems to confirm the guidance of ADC values as a sensitive marker of radio-resistance.

2. RATIONAL OF THE STUDY

Relapsed GBMs have a life expectancy of a few months and reirradiation has been shown to be safe in terms of toxicity and effective in increasing OS. Our recent study Ciammella P. et al [94] reported a median survival of 9.5 months in patients affected by recurrent GBM and treated with stereotaxic radiotherapy with a total dose of 25 Gy in 5 consecutive sessions, in which the dose was prescribed at isodose of 70% with a homogeneous gradient towards the center of the target volume. The identification with functional imaging of specific areas with higher tumor cell density, and the possibility of accurately delivering, thanks to the most advanced therapy units, different doses to the different sub-volumes, can lead to an increase in the maximum deliverable dose. In the most aggressive areas (with a greater tumour killing effect), compared to lower doses in the areas with the lowest signal alteration. This dose selectivity should allow an increase in the efficacy of the therapy and therefore a hypothesized increase in local control, in the face of a radio-induced toxicity on surrounding healthy tissues almost comparable to that achieved with previous hypofractionated treatments [94]. In fact, delivering very high doses to the entire volume would lead to an excess of radio-induced necrosis within the regions irradiated with high doses, as well as the impossibility of minimizing the doses on healthy areas and / or on non-neoplastic critical areas, keeping them at internal dose range associated with minimum and acceptable toxicity levels. Since there are no studies that provide clear indications on the acute and late toxicity of irradiated healthy tissues, that have already been the subject of a first course of radiotherapy (STUPP), the choice of safety is the primary objective of the study.

3. STUDY POPULATION

Patients suffering from:

- GBM relapsed after standard treatment surgery-radio-chemotherapy or other therapeutic lines;

or

- relapses of anaplastic astrocytoma with clear radiological signs of change.

3.1 Inclusion criteria

All patients who meet the following criteria will be eligible:

- GBM relapsed after standard surgery-radio-chemotherapy treatment, or other therapeutic lines, or GBM secondary to anaplastic astrocytoma previously treated with

radio and chemotherapy or relapses of anaplastic astrocytoma with clear radiological signs of change.

- Age \geq 18 years.
- ECOG Performance Status 0-2, Performance Karnofsky Score $>$ 60.
- Written informed consent.
- Life expectancy $>$ 3 months.
- Availability of the patient to be followed for all phases of radiotherapy treatment and for the subsequent follow-up.

3.2 Exclusion criteria

- Patients with KPS $<$ 70%.
- Participation in other studies involving the administration of experimental drugs.
- Any concomitant medical or psychological conditions that could prevent participation in the study or that compromise the ability to provide informed consent.
- Pregnant or breastfeeding patients.

3.3 Methods of recruitment

- Patients belonging to the U.O. of Oncological Radiotherapy, Neurology and Oncology of the ASMN-IRCCS of Reggio Emilia are eligible for participation in the study. After evaluation of eligibility and acquisition of informed consent from the patient, each will be assigned a progressive number which will be used to identify the subject during all phases of the study.

4. OBJECTIVES

4.1 Primary objective

The study proposes the evaluation of the side effects of the proposed experimental radiotherapy treatment and the acceptability of the risk / benefit ratio.

Acute / subacute toxicity will be analysed with recording of adverse events (related or unrelated) reported during the entire treatment and within 3 months of its end.

4.2 Secondary objectives

- Evaluation of the response rate according to the RANO criteria.
- Incidence of treatment-related radionecrosis measured with advanced MRI sequences. More information on the methods followed in the study to make a correct discrimination between radionecrosis and tumor relapses are given in a specific Technical Annex.
- Evaluation (descriptive) of the Quality of Life assessed through EORTC QLQ-C30 and BN20 questionnaires.
- Evaluation (descriptive) of neuro-cognitive functions as indicated in the rest of the protocol.
- Overall survival (OS) assessment.

- Assessment of progression-free survival (PFS).

5. DESIGN OF THE STUDY

It is an exploratory, prospective, experimental, monocentric study.

Having identified a main safety endpoint (toxicity of radiotherapy treatment), and in the absence of data currently available from previous studies and useful for sample sizing, the sample is defined according to criteria of expediency and feasibility, (12 cases), with a design (3 cases + 3 cases + 6 cases) which provides for two intermediate safety assessment steps, one month after the end of treatment for the third and sixth patient. The study will be continued only in the event of a positive evaluation of both steps.

5.1 Duration of the study

54 months of which 51 for patient enrolment and 3 months for completion of treatment and evaluation of the response of the last patient enrolled.

For each individual patient, the treatment is considered completed if:

- a) the experimental radiotherapy treatment was completed;
- b) the first follow-up radiological evaluation (NMR) was performed;
- c) study treatment was prematurely discontinued due to lack of efficacy (documented disease progression) or unacceptable toxicity.

5.2 Early termination of the study

An internal board will evaluate according to the analysis plan defined in section 12.1 the continuation / interruption of the study in the protection of patients' interests, based on information on the safety of the cases already treated.

The study can be concluded early:

- if the nature and frequency of the events and / or any urgent safety measures are such that the risk and benefit ratio is not acceptable;
- if the number of patients who drop out of the study is such that it is difficult to complete the study.

6. TREATMENT PLAN

6.1 Radiotherapy treatment

The treatment envisaged by the protocol involves the planning and execution of a hypofractionated radiotherapy (RT) treatment, administered in 5 daily sessions, with modulated intensity technique and inhomogeneous distribution of the dose guided by diffusion images obtained with magnetic resonance imaging (MRI).

The radiotherapy treatment must begin within 7 days from the date of execution of the centering MRI which will include both the classic morphological sequences with administration of contrast medium and functional. In this regard, for more details see the Technical Annex.

In selected cases and according to clinical judgment, it will be possible to request a review of the anatomic-pathological and radiological findings.

6.2 Irradiation technique

For each patient, a radiotherapy treatment plan will be carried out with conformed techniques such as intensity modulated radiotherapy (IMRT) with dose redistribution: dose-painting [95 - 97]. More details are described in the Technical Annex.

6.3 Positioning and immobilization of the patient

The patient should be treated in the supine position. The arms should be placed along the body. The use of a knee support is recommended. The head will be immobilized with a thermoplastic mask and corresponding neck rest supports.

6.4 Planning CT and MRI

The planning CT should possibly be acquired with reduced layer thickness that contains the entire volume of the skull and the upper part of the shoulders. This is done to allow for the possibility of planning non-coplanar treatments.

The last follow-up MRI or radiotherapy centering is used for planning, consisting of standard and functional sequences, in addition to those that the neuroradiologist will have considered useful to better characterize the clinical case in question. More details in this regard are described in the Technical Annex.

6.5 Target volume and organs at risk (OAR)

The following target volumes should be identified [98]:

- Gross tumor volume (GTV): is defined using and combining conventional and advanced MR imaging sequences.
- Planning target volume (PTV). It is represented by the GTV with three-dimensional expansion between 0 and 5 mm according to the problems highlighted during the segmentation phase of the tumor. In this regard, for more details see the Technical Annex.

The following primary risk organs (OARs) must be identified:

- Optic nerves. The definition of the related planning risk volume is also suggested (PRV: organ at risk with three-dimensional expansion; this margin must be chosen taking into consideration the acquisition thickness of the centering CT and the chosen GTV-PTV expansion margin)
- Chiasma. The definition of the relative PRV is also suggested (organ at risk with three-dimensional expansion; this margin must be chosen taking into consideration the acquisition thickness of the centering CT and the chosen GTV-PTV expansion margin)
- Retinas. The definition of the relative PRV is also suggested (organ at risk with three-dimensional expansion; this margin must be chosen taking into consideration the acquisition thickness of the centering CT and the chosen GTV-PTV expansion margin).

Alternatively, the eyeballs can be outlined.

- Brain stem.
- The following secondary OARs must be identified:
- Lenses / Crystalline. The definition of the relative PRV is also suggested (organ at risk with three-dimensional expansion; this margin must be chosen taking into consideration the acquisition thickness of the centering CT and the chosen GTV-PTV expansion margin)
- Healthy brain tissue. It is represented by the volume of the brain minus the PTV.
- Ear cochlea. The definition of the relative PRV is also suggested (organ at risk with three-dimensional expansion; this margin must be chosen taking into consideration the acquisition thickness of the centering CT and the chosen GTV-PTV expansion margin).

6.6 Dose prescription

6.6.1 Target dose

The prescription dose provides for a minimum dose of 30 Gy (6 Gy per session) for the areas with the highest ADC, with a gradual increase in the dose for the areas with the lowest ADC. The maximum hypothesized dose is 50 Gy, with a dose of 10 Gy per session at no more than 1cc of irradiated brain tissue. For more details see the Technical Annex.

The treatment plan, however, will be developed according to the following order of priority:

1. Compliance with dose limits for primary OAR.
2. PTV coverage.
3. Compliance with dose limits for secondary OARs.

6.6.2 Dose limits for primary organs at risk

In the scientific literature there are various studies that indicate the dose limits of the organs at risk for cerebral radiotherapy treatments. However, these limitations concern therapies in which conventional fractionations and deliveries to non-irradiated brain tissue are used [bibliography of the studies proposed in Table 1, 98]. The few reports relating to retreatments [99, 100] are mainly addressed to other anatomical areas with poor indications for brain treatments (only the spinal cord and brainstem is mentioned), especially when it comes to hypofractionated techniques as in our case. In retreatments with hypo-fractionation the references refer exclusively to healthy brain tissue. Not being able to use well-coded literature indications, it was decided to refer to the few literature data as well as to the clinical history of our radiotherapy center.

In the work of Ciammella [94] the outcomes of conventional hypofractionated retreatments (5Gy x 5 sessions) on glioblastomas performed at the Reggio Emilia radiotherapy are described both in terms of efficacy and acute toxicity. Therefore the radiotherapist, in the evaluation phase of the dose-painting plans envisaged in this study, will be able to use as an indication and reference of the doses on critical organs estimated for a conventional hypofractionated plan. Since these doses are a function of the anatomical configuration of the patient, as well as of the conformation and position of the tumor recurrence, it is necessary to carry out planning for each individual patient belonging to the study. The final word regarding the clinical approval of the plan to be provided will still be up to the radiotherapist who will clinically evaluate the treatment plan. For more details see the Technical Annex.

6.6.3 Dose limits for secondary risk organs

Also for secondary risk organs, what has been indicated in the previous point (6.6.2) regarding the relative dose limits is valid.

6.7 Changes or suspension of treatment

During the treatment program, neurological toxicities will be evaluated according to criteria of the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE), version 4.0. The patient will be monitored for the appearance of adverse events for the entire duration of the study, including the follow-up period.

Events	Actions
a) Neurological toxicity \leq grade 2 responsive to supportive care b) Grade \leq 3 non-neurological toxicity	The RadioTherapy treatment continue
c) grade $>$ 3 neurological and non-neurological toxicity; d) grade \leq 2 neurological toxicity unresponsive to supportive care; e) disease progression during treatment; f) second medical opinion, for safety reasons; g) refusal of the patient to continue the treatment; h) need to start antineoplastic therapy.	The presence of at least one of the events determines the definitive suspension of the treatment. It also involves leaving the study in cases (e) and (h)

In case of grade \leq 2 neurological toxicity with response to adequate supportive therapy (steroids, mannitol, anticomitials) the radiotherapy treatment will continue.

6.8 Recommended concomitant therapies and permitted drugs

The use of anticomitial drugs will be allowed, as prophylaxis, if patients are already on antiepileptic therapy, as well as the continuation of steroid therapy if already in place. The following drugs and / or supportive therapies can be used, if clinically indicated:

- steroids;
- antiemetics;
- mannitol i.v .;
- anticomitial drugs.

6.9 Concomitant Therapies Prohibited

During the entire duration of the treatment it will not be allowed to use:

- antineoplastic drugs;
- other experimental drugs.

7. EXIT FROM THE STUDY AND SUSPENSION OF THE TREATMENT

7.1 Exit from the study

Should one of the following conditions occur, the patient should be considered off-study and will not be included in the study's end point analysis:

- a. refusal of the patient to continue the treatment (withdrawal of informed consent);
- b. violation of the inclusion / exclusion criteria.

7.2 Suspension of treatment

Treatment must be permanently suspended if one or more of the following situations occur:

- grade > 3 neurological and non-neurological toxicity;
- grade ≤ 2 neurological toxicity unresponsive to supportive care;
- disease progression during treatment;
- second medical opinion, for safety reasons;
- refusal of the patient to continue the treatment;
- need to start antineoplastic therapy.

Permanent discontinuation of treatment, in case of disease progression or unacceptable toxicity, will be considered as a therapy failure, therefore patients will be included in the study's end-point analysis.

7.3 Evaluations in case of suspension of treatment

Patients who discontinue treatment for reasons other than death and withdrawal of consent will be evaluated with a follow-up visit within 7 days of discontinuing treatment. Patients who suspend the treatment, envisaged by the protocol, will receive medical treatment as required by the clinical practice of the center.

8. EVALUATION PROGRAM

8.1 Baseline assessment before the start of radiotherapy treatment

8.1.1 Mandatory assessments:

- general and neurological examination. A palliative doctor will be supported by the radiotherapist.
- Detection of vital parameters and anthropometric indices.
- Definition of the ECOG and Karnofsky indices.
- Quality of life assessment through EORTC QLQ-C30 and BN20 questionnaires.

8.1.2 Optional assessments based on tumor site:

- neurological visit.

- Endocrinological examination with hormonal profile (FT3, FT4, TSH, FSH, LH, Estradiol (E2) if female, testosterone if male, prolactin, cortisol, ACTH, GH).
- Otolaryngological examination with complete audiometric examination (tonal and vocal).
- Eye examination with neuro-ophthalmological evaluation.
- PET with labeled amino acids or with membrane metabolism tracers.
- Other examinations and / or specialist visits depending on the particular conditions of the patient.

8.2 Evaluation at the end of the radiotherapy treatment

Clinical visit at the end of radiotherapy:

- General and neurological examination. A palliative doctor will be assisted by the radiotherapist.
- Detection of vital parameters and anthropometric indices.
- Definition of the ECOG and Karnofsky indices.
- Detection of acute toxicity via CTCAE version 4.0 scale.

8.3 Follow-up evaluation

Clinical visits at 7 days, 15 days, 30 days, 60 days and 90 days from the end of radiotherapy (RT).

8.3.1 Mandatory assessments:

- General and neurological examination. A palliative doctor will be supported by the radiotherapist.
- Detection of vital parameters and anthropometric indices.
- Definition of the ECOG and Karnofsky indices.
- Detection of acute / subacute / late toxicity by CTCAE version 4.0 scale.
- Quality of life assessment through EORTC QLQ-C30 and BN20 questionnaires (at 1 and 3 months).
- MRI of the brain before and after m.d.c. paramagnetic (1 month and 3 months from the end of RT).

8.3.2 Optional assessments based on tumor site or patient's particular condition:

- Additional specialist visits (endocrinology, ophthalmology, etc.) according to medical indications.

9. OUTCOMES EVALUATION PLAN

The response to radiotherapy treatment will be evaluated according to the RANO criteria (Technical Annex) with morphological and functional (multiparametric) brain MRI performed at 1 month after the end of radiotherapy and then after 3 months.

The toxicity assessment will be made with reference to the International Common Toxicity Criteria for Adverse Event (CTCAE) - version 4.0 (The complete document is available on the website of the National Institute of Health

The response to radiotherapy treatment will be evaluated according to the RANO criteria (Annex) with morphological and functional (multiparametric) brain MRI performed at 1 month after the end of radiotherapy and after 3 months.

The toxicity assessment will be made with reference to the International Common Toxicity Criteria for Adverse Event (CTCAE) - version 4.0 (The complete document is available on the National Institute of Health (NIH) website at the following address: <http://ctep.cancer.gov/reporting/ctc.html>), excluding neurological adverse events of grade ≤ 2 that responded adequately to supportive care.

Safety and tolerability will be defined for:

- type, frequency, severity and relationship of adverse events to treatment;
- number of subjects who prematurely discontinued treatment due to any adverse event;
- frequency of clinically significant changes and / or laboratory tests.

All patients who have started radiation treatment will be considered for analysis, until the completion of the study (i.e. until the final visit at the end of the entire program for patients who complete the treatment) or until the scheduled follow-up visit in case of premature suspension of the study. Patients will be instructed by the Investigator on the need to report any adverse events that may occur during the study.

10. SAFETY ASSESSMENTS OF RADIOTHERAPY

The investigator will report all adverse events, serious and non-serious, related and unrelated within the CRF, with the exception of:

1. Any medical conditions of the patient present at baseline before the start of study treatment. Worsening of these conditions during the study will instead be recorded as an adverse event.
2. Abnormal laboratory values, unless they lead to exit from the study, a specific treatment, modification or interruption of study treatment, other therapeutic interventions or clinically important in the opinion of the Investigator. If the laboratory parameter is part of a pathological state, only the diagnosis of the same must be reported as AE within the CRF. If possible, the laboratory abnormality should be recorded as a diagnosis and not simply as an abnormal laboratory result.

10.1 Evaluation of adverse events from radiotherapy

An adverse event (AE) is any adverse clinical event that occurs in a patient or clinical trial subject receiving treatment and which does not necessarily have a causal relationship to that treatment. An adverse event can occur in the treatment period and in the follow-up period.

An adverse event is defined as SERIOUS (SAE) if it has:

- lethal outcome;
- endangers the patient's life (it means that in the Investigator's opinion the patient is at immediate risk of death);
- imposes a hospitalization or extends the hospitalization in progress following the procedures provided for in the protocol;
- involves severe or prolonged disability or incapacity, a congenital anomaly or malformation or a birth defect;
- may endanger the subject or require intervention to prevent one of the above situations.

Within the protocol, hospitalizations already planned before the start of the protocol or as parts of the protocol, hospitalizations lasting less than 24 hours or occurring in the absence of an adverse event will not be considered serious adverse events.

For each adverse event (AE), the Investigator will provide information on the nature of the event, severity, start and end date, actions taken, causation with the treatment, seriousness and outcome.

10.1.1 Severity / Severity of an event

The Investigator will have to evaluate the severity of the event on the International Common Toxicity Criteria for Adverse Event (CTCAE) classification - version 4.0, graduated on a scale from 1 to 5. In case of an event not included in the International Common Toxicity Criteria for Adverse toxicity scale Event (CTCAE) - version 4.0, the following scale will be used to indicate the severity of an event.

The severity of adverse events not listed in this classification will be assessed on the basis of the following levels:

- mild: the adverse event is easily tolerated by the subject, causing minimal discomfort and no interference with daily activities and does not require specific treatments;
- moderate: the adverse event interferes with normal daily activities but improves following therapeutic intervention;
- severe: the adverse event prevents normal daily activities and requires specific therapeutic intervention;
- life risk: immediate risk that requires hospitalization and clinical intervention;
- death.

10.1.2 Causation of an event

The Investigator is responsible for evaluating a causal link between the adverse event and the treatment, regardless of the doses administered, according to the following definitions and with respect to the information contained in the study documentation:

- not related: when the event is not considered related to the treatment (if the event is easily explained by the clinical situation, underlying diseases, concomitant therapies, specific procedures or if there is no temporal correlation);
- correlated (or possible correlation): when there are facts (evidence) or arguments that indicate or suggest a causal link (temporal relationship, nature of the event, dependence of the nature of the event on the suspension / resumption of treatment, unlikely relationship with concomitants, underlying pathologies or any procedures) between the event and the treatment.

10.1.3 Outcome

The Investigator must indicate the action taken due to an adverse event in terms of:

- continuation of treatment;
- interruption of treatment;

indicating whether additional interventions not provided for in the protocol were necessary for the management of the event. All adverse events that did not lead to the interruption of the subject's participation in the study must be monitored until resolution of the event, partial resolution, aggravation or death (due to the event itself).

The Investigator is responsible for an adequate clinical follow-up of the event until resolution, stabilization of the event, patient death or loss of the patient at follow-up. This may mean that in some circumstances the follow-up continues even after the end of the study.

10.2 Recording an Adverse Event

The Investigator must record any adverse events that occur during the treatment period and thereafter, up to the study closing visit in the CRF and in the relevant health records.

A completely resolved AE that recurs or increases in severity will have to be recorded as a new event within the CRF.

A description of the event will be reported with medical terms, date of presentation and resolution, severity, causation with respect to the treatment, actions taken, seriousness of the event. All the measures required for the management of the adverse event must be recorded in the reference health documentation.

If an AE is considered serious, the AE section within the CRF must be completed.

10.3 Intermediate safety reports

The investigator will produce a safety report as per the analysis plan defined in paragraph 12.1 and upon request to be submitted to the internal board and / or the Ethics Committee.

11. SURVEILLANCE ON MEDICAL DEVICES

Since the medical devices under study are CE marked and used in accordance with the intended use indicated by the Manufacturer, the surveillance will be carried out in accordance with the provisions for post-market investigations (MEDDEV 2.12.1 - Guidelines on a medical devices vigilance system): the specific definitions of adverse event and reporting obligations will therefore be applied and in compliance with the company Procedure "Supervision of medical devices, active implantable medical devices, in vitro diagnostic devices" (PRDMVIG01 of 12/01/2015).

In accordance with the provisions of the Meddev 2.7-3 guideline (Guidelines on medical devices clinical investigations: serious adverse event reporting), any safety reports will be notified to the Ethics Committee.

11.1 Security Definitions

Accident definition: an accident / near miss is any malfunction or deterioration of the characteristics and / or performance as well as any deficiency in the labeling or in the instructions for use of a device which, directly or indirectly, may cause or have caused death or serious injury. worsening of the state of health of the patient or of a user or other people.

Any event that meets all three of the basic detection criteria listed below is considered an accident and must be reported to the National Competent Authority.

- **An event happened**

This also includes situations in which, following tests performed on the device or following the analysis of the information provided with the device or any other scientific information, factors emerge that could lead or have led to an event. Typical events include, but are not limited to:

- a. a malfunction or deterioration in characteristics or performance (a malfunction or deterioration must be interpreted as an inability of the device to operate in accordance with its intended use, even if the device is used according to the manufacturer's instructions);
- b. false positive or false negative test results that are outside the stated performance limits of the test;
- c. unexpected adverse reactions or unexpected side effects;
- d. interactions with other substances or products;
- e. degradation / destruction of the device;
- f. inappropriate therapy;

- g. lack of accuracy in labeling, instructions for use and / or promotional material. Lack of accuracy includes omissions and deficiencies. Omissions do not include the absence of information that should be well known to the intended user.
- **It is suspected that the manufacturer's medical device is a contributing cause of the accident**

In assessing the link between the device and the accident / missed accident, the Investigator or other healthcare professional must take into account:

- a. the opinion, based on available evidence, of health professionals;
 - b. evidence of similar incidents that occurred previously;
 - c. the results of the manufacturer's preliminary assessment;
 - d. other evidence held by the manufacturer;
 - e. if the judgment is difficult and in complex circumstances it must be assumed that the device may have caused or contributed to causing the accident and that the manufacturers have not paid due caution.
- **The event caused, or could have caused the death or serious deterioration of the state of health (of the patient, user or other person)**

Severe deterioration in health can mean:

- a. a serious illness;
- b. permanent impairment of a bodily function or permanent damage to a bodily structure;
- c. a condition that requires medical or surgical intervention to prevent cases (a) or (b);
- d. any indirect damage resulting from the onset of incorrect diagnostic results or IVD tests, when used in compliance with the manufacturer's instructions for use;
- e. fetus distress, fetus death, or any congenital anomalies or birth defects.

PLEASE NOTE: It should be noted that not all accidents involve a risk and / or cause death or serious deterioration of the state of health (of the patient, user or other person). The non-occurrence of this outcome may be due to other circumstances or the intervention of the healthcare professional. It is therefore sufficient that an accident associated with a device has occurred, and that the accident was such that, if it recurs, it could lead to death or serious deterioration of health (of the patient, user or other person).

11.2 Timing for reporting an Incident

The reporting of an accident by the Investigator or other health professionals must take place immediately after the occurrence of the accident / missed accident, to allow compliance with the transmission times to the Ministry, or within a maximum of 10 days.

The report must be sent signed by the Investigator to the contact person for the surveillance device (RAV) of Active Medical Devices and Health Equipment of CND Y and Z class, completing the report form (Attachment), available on the company intranet in a complete and legible way following the path: Portal Home> Medical Devices Company Commission> MEDICAL DEVICES SURVEILLANCE.

*Contact person for the supervision of the
Active Medical Devices and health equipment of CND Y and Z class*

Ing. Paolo Bottazzi

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For 10 days following the submission of the report, the medical device must remain available to the competent authority. After this deadline, the medical device subject to reporting must be entrusted to the RAV subject to agreements with the same for the times and methods of delivery.

11.3 Forwarding of the report to the Competent Authority and to the Ethics Committee

The RAV provides for the transmission of the complete Accident Form signed by the Investigator, together with a cover letter, to the Ministry of Health at Office V of the General Directorate of Medical Devices and Pharmaceutical Service (DGDMF). This documentation will also be sent to the local Ethics Committee.

The RAV will transmit the appropriate feedback information to the reporter, the PI of the study and the Local Ethics Committee.

11.4 Urgent security measures

Any communications relating to safety notices and / or recall on medical devices in use within the study, communicated by the Manufacturer and / or Competent Authority and received by the RAV, will be sent to the IP of the study and to the provincial Ethics Committee and to the internal board (for safety assessment).

12. STATISTICAL ASPECTS

12.1 The champion

The study is proposed as an exploratory, prospective, experimental and monocentric study.

Regarding the main endpoint (toxicity of the proposed radiotherapy treatment), there are no data currently available from previous studies. In the absence of criteria useful for the sizing of the

sample in formal terms, the sample (12 cases) is defined according to criteria of opportunity and feasibility, with two steps of evaluation of the outcome as follows:

- after the first 3 patients enrolled and treated with the proposed radiotherapy scheme, there will be a 1 month stop in the enrollment of subsequent patients, in which tolerance and the occurrence of serious adverse events will be analyzed.
- At the end of the month, if none of the patients treated shows toxicity attributable to radiation treatment > 3, the enrollment of the next 3 patients will continue with a further stop in enrollment for another month.
- If these patients, together with the 3 previously treated, do not have serious adverse events induced by radiation treatment, the enrollment of the last 6 patients will continue.

All patients in the study will be followed, for the primary endpoint of the study, for at least 3 months from enrollment, or until disease progression or death from another cause.

At the end of each intermediate step, the investigator will produce a report on the safety of the cases already treated which will be subjected to the evaluation of an internal board, composed of the Health Director, the Scientific Director and the Legal Doctor of the ASMN-IRCCS of Reggio Emilia, who will express on the continuation / interruption of the study and subsequently notified to the EC.

The internal board will also evaluate any urgent safety measures (as defined in paragraph 11.4) transmitted by the RAV, expressing its opinion on the continuation / interruption of the study and subsequently notifying the EC of this opinion.

12.2 Analysis plan

By virtue of the exploratory nature of the study and the limited number of subjects, the data will be presented mainly through tables and lists. Confidence intervals for percentages will be calculated following the Clopper-Pearson method to properly take into account the limited number of subjects. OS and PFS will be graphed using the Kaplan-Meier method and briefly described in percentile terms. The analyses will be conducted with R, SAS System, or SPSS software on the basis of availability at the time of the analyzes and in the versions in use in that period

12.3 Data management

For the management of the study, a data collection form on the web platform (e-CRF) with personalized access credentials will be requested from the Company's IT Service (STIT), which meets regulatory requirements and guarantees privacy criteria in accordance with the law (Authorization of the Privacy Guarantor n.72 of 26 March 2012). All data will be collected and processed in codified form.

A paper draft of the CRF will be provided to the Ethics Committee, which is being prepared.

Patients will be registered in the CRF, registration form, at the time of entry into the study (Signature of informed consent). All signed consents and patient screening and enrolment procedures must be kept in the study's Trial Master File.

13. ETHICAL AND AUTHORIZATION ASPECTS

13.1 General aspects

The study will be activated only after approval by the Local Ethics Committee / competent Authorities, and any communications to the Ministry of Health and will be conducted in accordance with this protocol and any amendments introduced.

The protocol has been written and the study will be conducted in accordance with the ethical principles of the Declaration of Helsinki (<http://www.wma.net/e/policy/b3.htm>) and ICH Harmonized Tripartite Guideline for Good Clinical Practice (<http://www.ifpma.org/pdfifpma/e6.pdf>) and UNI ISO 14155 (2012) (Clinical investigation of medical devices for human subject-GCP) and following the guidelines on the medical device vigilance system Meddev 2.12-1.

The study documentation will be collected and stored at the participating centers and in its entirety by the coordinating center of the study (Medical Physics Service, ASMN-IRCCS of Reggio Emilia) and will be stored according to regulatory indications.

All documentation must be made available for any inspections required by regulatory authorities, during or after the conclusion of the study, and for planned monitoring activities.

Each patient eligible for treatment will be adequately informed by the specialist on the purposes of the study, the expectations of the proposed experimental intervention, the objectives and methods of conducting the study and must sign a specific consent for the study and data processing.

Each patient has the right to withdraw their membership at any time without having to justify the choice; from that moment on his data will no longer contribute to the study dataset and documented in the patient's medical record.

13.2 Insurance aspects

The study in question is defined as a non-profit study, aimed at improving clinical practice as an integral part of health care; no medicine is envisaged and the study has no industrial purpose. In these cases, the legislation decrees that as a guarantee for any damage caused to the patient to cover the risks associated with the experimental treatment not already documented, it is possible to take advantage of the coverage already provided for the assistance activity which is also extended to the experimentation activities.

The information on the risks known to the treatment and on the company insurance policy covering the study must be specifically provided to the patient in the information documentation, before signing the consent.

13.3 Monitoring and Quality Control (QC)

Through an electronic e-CRF set up on a web platform by the IT Service of the ASMN-IRCCS company, of Reggio Emilia (promoter of the study), the registration of patients entering the study and the availability of clinical data will be guaranteed with continuity.

The Clinical Studies and Statistics Office will propose a monitoring program suitable for the type of study in accordance with the company SOPs with centralized monitoring actions, off site and on site, managed by adequately trained staff.

In the event of early termination of treatment, patients will still be monitored for the entire duration of the study or for any documented disease progression, death or exit from follow-up.

13.4 Responsibility and publication policy

The study is independent and promoted by the IRCCS-ASMN Hospital which will manage the conduct of the study, the collection and analysis of data and its dissemination.

The submission of articles to scientific journals and / or the presentation of papers at national / international scientific conferences will be attended by the experimenters interested in the study and those who participated in the development and drafting of the project.

The results will be published and / or presented at scientific conferences even in the event of an early interruption of the study.

14. BIBLIOGRAPHY

1. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005; 352 (10): 987-96.
2. Stupp R, Hegi ME, Mason WP et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009. Mar 6
3. Central Brain Tumor Registry of the United States. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2004-2007. www.cbtrus.org. Accessed June 28, 2012
4. Nieder C, Grosu AL, Molls M. A comparison of treatment results for recurrent malignant gliomas. *Cancer Treatment Reviews* 2000; 26:397-409
5. Ammirati M, Galicich JH, Arbit E, Liao Y. Reoperation in the treatment of recurrent intracranial malignant gliomas. *Neurosurgery* 1987; 21:607-14
6. Manikandan A, Sarkar B, Holla R, Vivek TR, Sujatha N. Quality assurance of dynamic parameters in volumetric modulated arc therapy. *Br J Radiol* 2012; 85: 1002-1010.

7. Richmond ND, Pilling KE, Peedell C, Shakespeare D, Walker CP. Positioning accuracy for lung stereotactic body radiotherapy patients determined by on-treatment cone-beam CT imaging. *Br J Radiol* 2012; 85: 819-823.
8. Brandes AA, Tosoni A, Cavallo G, et al. Temozolomide 3 weeks on and 1 week off as first-line therapy for recurrent glioblastoma: phase II study from Gruppo Italiano Cooperativo di Neuro-oncologia (GICNO). *Br J Cancer*. 2006;95(9):1155-1160
9. Hammond A, Norden AD, Lesser GJ, et al. Phase II study of dose-intense temozolomide in recurrent glioblastoma. *J Clin Oncol*. 2011;29:2038
10. Brandes AA, Franceschi E, Tosoni A, Bartolini S, Bacci A, Agati R, et al. O(6)-methylguanine DNA-methyltransferase methylation status can change between first surgery for newly diagnosed glioblastoma and second surgery for recurrence: clinical implications. *Neuro-Oncology* 2010; 12: 283-8
11. Schultheiss TE, Kun LE, Ang KK, et al. Radiation response of the central nervous system. *Int J Radiat Oncol Biol Phys* 1995; 31:1093-112
12. Veninga T, Langendijk HA, Slotman BJ, et al. Reirradiation of primary brain tumors: survival, clinical response and prognostic factors. *Radiother Oncol* 2001;59:127-37
13. Barbagallo GM, Jenkinson MD, Brodbelt AR. Recurrent Glioblastoma multiforme- when should we reoperate? *Br J Neurosurg*. 2008;22(3):452-455
14. Young B, Oldfield EH, Markesbery WR, Haack D, Tibbs Pa, McCoombs P, et al. Reoperation for glioblastoma. *Journal of Neurosurgery* 1981; 55:917-21
15. Barker 2nd FG, Chang SM, Gutin PH, Malec MK, McDermott MW, Prados MD, et al. Survival and functional status after resection of recurrent glioblastoma multiforme. *Neurosurgery* 1998; 42:709-20
16. Barker 2nd FG, Chang SM, Gutin PH, Malec MK, McDermott MW, Prados MD, et al. Survival and functional status after resection of recurrent glioblastoma multiforme. *Neurosurgery* 1998; 42:709-20
17. Coombs SE, Debus J, Shulz-Ertner D. Radiotherapeutic alternatives for previously irradiated recurrent gliomas. *BMC Cancer*. 2007;7:167
18. Hall WA, Djalilian HR, Sperduto PW, et al. Stereotactic radiosurgery for recurrent malignant gliomas. *J Clin Oncol*. 1995; 13:1642-1648
19. Cho KH, Hall WA, Gerbi BJ, Higgins PD, et al. Single dose versus fractionated stereotactic radiotherapy for recurrent high-grade gliomas. *Int J Radiat Oncol Biol Phys*.1999; 45: 1133-1141
20. Combs SE, Gutwein S, Thilman CH, et al. Stereotactically guided fractionated reirradiation in recurrent glioblastoma multiforme. *J Neurooncol*. 2005; 74:167-171
21. Combs SE, Thilman CH, Edler L, et al. Efficacy of fractionated stereotactic reirradiation in recurrent gliomas: long-term result in 172 patients treated in a single institution. *J Clin Oncol*. 2005; 23:8863-8869
22. Vordermark D, Kolbl O, Ruprecht K, et al. Hypofractionated stereotactic re-irradiation: treatment option in recurrent malignant glioma. *BMC Cancer*. 2005; 5:55

23. Lederman G, Wronski M, Arbit E, et al. Treatment of recurrent glioblastoma multiforme using fractionated stereotactic radiosurgery and concurrent paclitaxel. *Am J Clin Oncol.* 2000; 23:155-159
24. Arcicasa M, Roncadin M, Bidoli E, et al. Reirradiation and lomustine in patients with relapsed high-grade gliomas. *Int J Radiat Oncol Biol Phys.* 1999; 43:789-793
25. Combs SE, Bischof M, Welzel T, et al. Radiochemotherapy with temozolomide as re-irradiation using high precision fractionated stereotactic radiotherapy (FSRT) in patients with recurrent gliomas. *J Neurooncol.* 2008; 89:205-210
26. Minniti G, Armosini V, Salvati M, et al. Fractionated stereotactic reirradiation and concurrent temozolomide in patients with recurrent glioblastoma. *J Neurooncol.* 2011 Jul;103(3):683-91.
27. Bauman G.S., Sneed P.K., Wara W.M. et al. Reirradiation of primary CNS tumors. *Int. J. Radiat. Oncol. Bbiol. Phys.* 1996, 36,433-441
28. Kim H.K., Thorton A.F., Greenberg H.S., et al. Results of re-irradiation of primary intracranial neoplasms with three-dimensional conformal therapy. *Am. J. Clin. Oncol.* 1997, 20, 358-363.
29. Hayat K., Jones B., Bisbrown G. Et al. Retreatment of patients with intracranial gliomas by external beam radiotherapy and cytotoxic chemotherapy. *Clin. Oncol. (R. Coll. Radiol.)* 1997, 9, 158-163.
30. Nieder C., Nestle U., Ketter R. et al. Hyperfractionated and accelerated-hyperfractionated radiotherapy for glioblastoma multiforme. *Radiat. Oncol. Investig.* 1999, 7, 36-41.
31. Henke G., Paulsen F., Steinbach J.P. et al. Hypofractionated reirradiation for recurrent malignant glioma. *Strahlenther. Onkol.* 2009, 185, 113-119.
32. Osman M.A., et al. Phase II trial of temozolomide and reirradiation using conformal 3D-radiotherapy in recurrent brain gliomas. *Ann. Transl. Med.* 2014, 2(5):44
33. Grosu A.L., Weber W.A., Franz M. et al. Reirradiation of recurrent high-grade gliomas using amino acid PET (SPECT)/CT/MRI image fusion to determine gross tumor volume for stereotactic fractionated radiotherapy. *Int. J. Radiat. Oncol. Biol. Phys.* 2005, 63, 511-519.
34. Laing R.W., Warrington A.P., Graham J. et al. Efficacy and toxicity of fractionated stereotactic radiotherapy in the treatment of recurrent gliomas (phase I/II study). *Radiother. Oncol.* 1993, 27, 22-29.
35. Selch M.T., DeSalles A.A.F., Solberg T.D., Wallace R.E. et al. Hypofractionated stereotactic radiotherapy for Recurrent Malignant Gliomas. *J. Radiosurg.* 2000, 3, 3-12.
36. Wurm R.E., Kuczer D.A., Schlenger L. et al. Hypofractionated stereotactic radiosurgery combined with topotecan in recurrent malignant glioma. *Int. J. Radiat. Oncol. Biol. Phys.* 2006, 66, S26-S32.
37. Kohshi K, Yamamoto H, Nakahara A, et al. Fractionated stereotactic radiotherapy using gamma unit after hyperbaric oxygenation on recurrent high-grade gliomas. *J Neurooncol* 2007;82:297–303.
38. Ciammella P, Podgornii A., Galeandro M., et al. Hypofractionated stereotactic radiation therapy for recurrent glioblastoma: single institutional experience. *Radiat Oncol.* 2013, 25: 8:222.

39. Ernst-Stecken A, Ganslandt O, Lambrecht U, Sauer R, Grabenbauer G. Survival and quality of life after hypofractionated stereotactic radiotherapy for recurrent malignant glioma. *J Neurooncol*, 2007; 81:287-294
40. Fokas E, Wacker U, Gross MW, Henzel M, Encheva E, Engenhart-Cabillic R. Hypofractionated stereotactic reirradiation of recurrent glioblastomas: a beneficial treatment option after high-dose radiotherapy? *Strahlenther Onkol*. 2009; 185:235-240
41. Schwer A.L., Damek D.M., Kavanagh B.D. et al. A phase I dose-escalation study of fractionated stereotactic radiosurgery in combination with gefitinib in patients with recurrent malignant gliomas. *Int. J. Radiat. Oncol. Biol. Phys.* 2008, 70, 993-1001.
42. Patel M., Siddiqui F., Jin J.Y. et al. Salvage reirradiation for recurrent glioblastoma with radiosurgery: radiographic response and improved survival. *J. Neurooncol*. 2009, 92, 185-191.
43. Gutin P.H., Iwamoto F.M., Beal K. et al. Safety and efficacy of bevacizumab with hypofractionated stereotactic irradiation for recurrent malignant gliomas. *Int. J. Radiat. Oncol. Biol. Phys.* 2008, 75, 156-163.
44. Fogh SE, Anrews DW, Glass J, Curran W, Glass C, Champ C, Evans JJ, Hyslop T, Pequignot E, Downes B, Comber E, Maltenfort M, Dicker AP, Werner-Wasik M. Hypofractionated stereotactic radiation therapy: an effective therapy for recurrent high-grade gliomas. *J Clin Oncol*, 2010; 28:3048-3053
45. Maier-Hauff K., Ulrich F., Nestler D., et al. Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. *J. Neurooncol*. 2011, 103, 317-324.
46. Villavicencio A.T., Burneikiene S., Romanelli P. et al. Survival following stereotactic radiosurgery for newly diagnosed and recurrent glioblastoma multiforme: A multi center experience. *Neurosurg. Rev.* 2009, 32, 417-424.
47. Torcuator R.G., Thind R., Patel M. et al. The role of salvage reirradiation for malignant gliomas that progress on bevacizumab. *J. Neurooncol*. 2010, 97, 401-407.
48. Shepherd SF, Laing RW, Cosgrove VP, Warrington AP, Hines F, Ashley SE, Brada M: Hypofractionated stereotactic radiotherapy in the management of recurrent glioma. *Int J Radiat Oncol Biol Phys* 1997, 37:393-398.
49. Glass J, Silverman CL, Axelrod R, Corn BW, Andrews DW: Fractionated stereotactic radiotherapy with cis-platinum radiosensitization in the treatment of recurrent, progressive or persistent malignant astrocytoma. *Am J Clin Oncol* 1997, 20:226-229.
50. Hudes RS, Corn BW, Werner-Wasik M, Andrews D, Rosenstock J, Thoron L, Downes B, Curran WJ: A phase I dose escalation study of hypofractionated stereotactic radiotherapy as salvage therapy for persistent or recurrent malignant glioma. *Int J Radiat Oncol Biol Phys* 1999, 43:291-298
51. Voynov G, Kaufman S, Hong T, Pinkerton A, Simon R, Dowsett R: Treatment of recurrent malignant gliomas with stereotactic intensity modulated radiation therapy. *Am J Clin Oncol* 2002, 25:606-611.
52. Wuthrick EJ., Curran WJ., Camphausen K.. et al. A pilot study of hypofractionated stereotactic

- radiation therapy and sunitinib in previously irradiated patients with recurrent high-grade glioma. *Int. J. Radiat. Oncol. Biol. Phys.* 2014, 90(2):369-75.
53. Alexander E., Loffler J.S. Radiosurgery using a modified linear accelerator. *Neurosurg. Clin. N. Am.* 1992, 3, 167-190.
 54. Chamberlain M.C., Barba D., Kormanik R.N., et al. Stereotactic radiosurgery for recurrent gliomas. *Cancer* 1994, 74, 1342-1347.
 55. Shrieve D.C., Alexander E., Wen P.Y., et al. Comparison of stereotactic radiosurgery and brachytherapy in the treatment of recurrent glioblastoma multiforme. *Neurosurgery* 1995, 36, 275-284.
 56. Greenspoon JN, Sharieff W, Hirte H et al. Fractionated stereotactic radiosurgery with concurrent temozolomide chemotherapy for locally recurrent glioblastoma multiforme: a prospective cohort study. *Onco Targets Ther.* 2014 Mar 24;7:485-90.
 57. Shrieve D.C., Alexander E., Wen P.Y., et al. Comparison of stereotactic radiosurgery and brachytherapy in the treatment of recurrent glioblastoma multiforme. *Neurosurgery* 1995, 36, 275-284.
 58. Larson D.A., Gutin P.H., McDermott M. et al. Gamma knife for glioma: Selection factors and survival. *Int. J. Radiat. Oncol. Biol. Phys.* 1996, 36, 1045-1053.
 59. Kondziolka D., Flickinger J., Jonh C. et al. Survival benefit of stereotactic radiosurgery for patients with malignant glial neoplasm. *Neurosurgery* 1997, 41, 776-785.
 60. Park J.L., Suh J.H., Barnett G.H., Reddy C.S. et al. Survival after stereotactic radiosurgery for recurrent glioblastoma multiforme. *J. Radiosurg.* 2000, 3, 169-175.
 61. Larson D.A., Prodos M., Lamborn K.R., et al. Phase II study of high central dose gamma knife radiosurgery and marimastat in patients with recurrent malignant glioma. *Int. J. Radiat. Oncol. Biol. Phys.* 2002, 54, 1397-1304.
 62. Coombs S.E., Widmer V., Thilmann C. et al. Stereotactic radiosurgery (SRS). Treatment option for recurrent glioblastoma multiforme (GBM). *Cancer* 2005, 104, 2168-2173.
 63. Hsieh P.C., Chandler J.P., Bhangoo S. et al. Adjuvant gamma knife stereotactic radiosurgery at the time of tumor progression potentially improves survival for patients with glioblastoma multiforme. *Neurosurgery* 2005, 57, 684-691.
 64. Mahajan A., McCutcheon I.E., Suki D. et al. Case-control study of stereotactic radiosurgery for recurrent glioblastoma multiforme. *J. Neurosurg.* 2005, 103, 210-217.
 65. Kong D.S., Lee J.I., Park K. et al. Efficacy of stereotactic radiosurgery as a salvage treatment for recurrent malignant gliomas. *Cancer* 2008, 112, 2046-2051.
 66. Biswas T., Okunieff P., Schell M.C. et al. Stereotactic radiosurgery for glioblastoma: Retrospective analysis. *Radiat. Oncol.* 2009, 4, 11.
 67. Maranzano E., Anselmo P. Casale M. et al. Treatment of recurrent glioblastoma with stereotactic radiotherapy: Long-term results of a mono-institutional trial. *Tumori* 2011, 97, 56-61.
 68. Greenspoon JN, Sharieff W, Hirte H et al. Fractionated stereotactic radiosurgery with concurrent temozolomide chemotherapy for locally recurrent glioblastoma multiforme: a

- prospective cohort study. *Onco Targets Ther.* 2014 Mar 24;7:485-90.
69. Martínez-Carrillo M, Tovar-Martín I, Zurita-Herrera M et al. Salvage radiosurgery for selected patients with recurrent malignant gliomas. *Biomed Res Int.* 2014;2014:657953.
 70. Souhami L, Seiferheld D., Brachman D. et al. Randomized compariso of stereotactic radiosurgery followed by conventional radiotherapy with carmustine to conventional radiotherapy with carmustine for patients with glioblastoma multiforme: report of Radiation Therapy Oncology Group 93-05 protocol. *Int. J. Radiat. Oncol. Biol. Phys.* 2004, 60, 3: 853-860.
 71. Dadoo E, Huffmann B, Peredo I et al. Increased survival using delayed gamma knife radiosurgery for recurrent high-grade glioma: a feasibility study. *World Neurosurg.* 2014 Nov;82(5):e623-32.
 72. Pourtian N, Crowley W., Sherman J.H. Et al. Gamma Knife radiosurgery after radiation therapy as an adjunctive treatment for glioblastoma. *J Neuro-Oncol.* 2009, 94, 409-418.
 73. Masui K., Cloughesy T.F., Mischel P.S. Et al. Review: molecular pathology in adult high-grade gliomas: from molecular diagnostics to target therapies. *Neuropathology and applied neurobiology.* 2012, 38,3: 271-291.
 74. Cabrera AR, Cuneo KC, Desjardins A, et al. Concurrent stereotactic radiosurgery and bevacizumab in recurrent malignant gliomas: a prospective trial. *Int J Radiat Oncol Biol Phys.* 2013 Aug 1;86(5):873-9
 75. Isambert A, Dhermain F, Bidault F, et al. Evaluation of an atlas based automatic segmentation software for the delineation of brain organs at risk in a radiation therapy clinical context. *Radiother nOncol,* 2008,87:93-99.
 76. Metcalfe P, Liney GP, Holloway L, et al. The potential for an enhanced role for MRI in radiation-therapy treatment planning. *Technol Cancer Res Treat,* 2013,12:429-446.
 77. Holdsworth CH, Corwin D, Stewart RD, et al. Adaptive IMRT using a multiobjective evolutionary algorithm integrated with a diffusion invasion model of glioblastoma. *Phys Med Biol,* 2012,57:8271-
 78. Neal ML, Trister AD, Ahn S, et al. Response classification based on a minimal model of glioblastoma growth is prognostic for clinical outcomes and distinguishes progression from pseudoprogression. *Cancer Res,* 2013,73:2976-2986.
 79. McMillan KM, Rogers BP, Koay CG, Laird AR, Price RR, Meyerand ME. An objective method for combining multi-parametric MRI datasets to characterize malignant tumors. *Med Phys.* 2007 Mar;34(3):1053-61.
 80. Mabray MC, Barajas RF Jr, Cha S. Modern brain tumor imaging. *Brain Tumor Res Treat.* 2015 Apr;3(1):8-23.
 81. Bruzzone MG, D'Incerti L, Farina LL, Cuccarini V, Finocchiaro G. CT and MRI of brain tumors. *Q J Nucl Med Mol Imaging.* 2012 Apr;56(2):112-37.
 82. Horská A, Barker PB. Imaging of brain tumors: MR spectroscopy and metabolic imaging. *Neuroimaging Clin N Am.* 2010 Aug;20(3):293-310.
 83. Lee SK. Diffusion tensor and perfusion imaging of brain tumors in high-field MR imaging. *Neuroimaging Clin N Am.* 2012 May;22(2):123-34.

84. Gerstner ER, Sorensen AG. Diffusion and diffusion tensor imaging in brain cancer. *Semin Radiat Oncol.* 2011 Apr;21(2):141-6.
85. Ginat DT, Mangla R, Yeane G, Wang HZ. Correlation of diffusion and perfusion MRI with Ki-67 in high-grade meningiomas. *AJR Am J Roentgenol.* 2010 Dec;195(6):1391-5.
86. Chen L, Zhang J, Chen Y, Wang W, Zhou X, Yan X, Wang J. Relationship between apparent diffusion coefficient and tumour cellularity in lung cancer. *PLoS One.* 2014 Jun 11; 9(6):e99865.
87. Schnapauff D, Zeile M, Niederhagen MB, Fleige B, Tunn PU, Hamm B, Dudeck O. Diffusion-weighted echo-planar magnetic resonance imaging for the assessment of tumor cellularity in patients with soft-tissue sarcomas. *J Magn Reson Imaging.* 2009 Jun;29(6):1355-9.
88. Chen L, Liu M, Bao J, Xia Y, Zhang J, Zhang L, Huang X, Wang J. The correlation between apparent diffusion coefficient and tumor cellularity in patients: a meta-analysis. *PLoS One.* 2013 Nov 11;8(11):e79008.
89. Matsumoto Y, Kuroda M, Matsuya R, Kato H, Shibuya K, Oita M, Kawabe A, Matsuzaki H, Asaumi J, Murakami J, Katashima K, Ashida M, Sasaki T, Sei T, Kanazawa S, Mimura S, Oono S, Kitayama T, Tahara S, Inamura K. In vitro experimental study of the relationship between the apparent diffusion coefficient and changes in cellularity and cell morphology. *Oncol Rep.* 2009 Sep;22(3):641-8.
90. Alexiou GA, Zikou A, Tsiouris S, Goussia A, Kosta P, Papadopoulos A, Voulgaris S, Kyritsis AP, Fotopoulos AD, Argyropoulou MI. Correlation of diffusion tensor, dynamic susceptibility contrast MRI and (99m)Tc-Tetrofosmin brain SPECT with tumour grade and Ki-67 immunohistochemistry in glioma. *Clin Neurol Neurosurg.* 2014 Jan;116:41-5. doi: 10.1016.
91. Baker F1, Sanger L. The density of clonogenic cells in human solid tumors. *Int J Cell Cloning.* 1991 Mar;9(2):155-65.
92. Maani R, Yang YH, Kalra S. Voxel-based texture analysis of the brain. *PLoS One.* 2015 Mar 10;10(3): e0117759.
93. Alobaidli S, McQuaid S, South C, Prakash V, Evans P, Nisbet A. The role of texture analysis in imaging as an outcome predictor and potential tool in radiotherapy treatment planning. *Br J Radiol.* 2014 Oct;87(1042):20140369.
94. Ciammella P, Podgornii A, Galeandro M, D'Abbiere N, Pisanello A, Botti A, Cagni E, Iori M, Iotti C. Hypofractionated stereotactic radiation therapy for recurrent glioblastoma: single institutional experience. *Radiat Oncol.* 2013 Sep 25;8:222
95. Supiot S, Lisbona A, Paris F, Azria D, Fenoglietto P. ["Dose-painting": myth or reality?]. *Cancer Radiother.* 2010 Oct;14(6-7):554-62.
96. Ken S, Vieilleigne L, Franceries X, Simon L, Supper C, Lotterie JA, Filleron T, Lubrano V, Berry I, Cassol E, Delannes M, Celsis P, Cohen-Jonathan EM, Laprie A. Integration method of 3D MR spectroscopy into treatment planning system for glioblastoma IMRT dose painting with integrated simultaneous boost. *Radiat Oncol.* 2013 Jan 2;8:1.
97. Rickhey M, Koelbl O, Eilles C, Bogner L. A biologically adapted dose-escalation approach, demonstrated for 18F-FET-PET in brain tumors. *Strahlenther Onkol.* 2008 Oct;184(10):536-42.

98. Nguyen D, Rwigema JC, Yu VY, Kaprealian T, Kupelian P, Selch M, Lee P, Low DA, Sheng K. Feasibility of extreme dose escalation for glioblastoma multiforme using 4π radiotherapy. *Radiat Oncol*. 2014 Nov 7;9:239.
99. Chojnacka M, Skowrońska-Gardas A, Pędziwiatr K, Morawska-Kaczyńska M, Perek M, Perek D. Reirradiation of relapsed brain tumors in children. *Rep Pract Oncol Radiother*. 2011 Nov 13;17(1):32-7.
100. Sminia P, Mayer R. External beam radiotherapy of recurrent glioma: radiation tolerance of the human brain. *Cancers (Basel)*. 2012 Apr 5;4(2):379-99.