Universitat de Girona
Facultat de Medicina

EFFECT OF CANNABINOIDS ON THE SURVIVAL OF GLIOBLASTOMA

A PHASE II MULTICENTER RANDOMIZED CLINICAL TRIAL

FINAL DEGREE PROJECT

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1. ABSTRACT

BACKGROUND: glioblastoma multiforme is the most common primary malignant tumor of the central nervous system in adults. In our environment, the incidence is 4.17 (95% CI 3.80-4.57) cases per 100.000 inhabitants/year, with a global survival per year of 61%, 26.5% at two years and less than 5% after 5 years. It is considered an "orphan" treatment disease; the treatment is very palliative and seeks to control tumor growth and not progress. This has not changed since 2005, the year in which Stupp, R., et al. showed that the concomitance of chemotherapy and radiotherapy after surgery increased survival. In preclinical and clinical studies it has been seen that cannabinoids have antitumor effects, altering the growth, invasion and angiogenesis of cancer cells, which could be an advance in the prognosis of this tumor. An increase in endogenous cannabinoid levels has been seen in patients with glioblastoma as well as increased expression of cannabinoid receptors.

OBJECTIVE: demonstrate that the one-year overall survival rate increases at least 20% by adding cannabinoids to the base treatment (Stupp regimen) with good tolerance, in addition to delaying the time to progression, compared to those patients who only receive the standard treatment.

DESIGN: the study will be a multicenter, longitudinal, prospective, double-blind, randomized and controlled clinical trial. It will be carried out in the 3 cancer hospitals of Institut Català d'Oncologia. The one in Girona will be the coordinator center.

INTERVENTION AND METHODS: subjects of the study will be those newly diagnosed glioblastoma patients who, after resection, begin medical treatment. They will be randomly divided into two groups: group A (n = 30), patients will be treated with the classic scheme plus concomitant cannabinoids during chemotherapy and radiotherapy until progression; in group B (n = 29), patients will receive the same Stupp scheme plus concomitant placebo treatment instead of cannabinoids. Recruitment of patients will last 12 months, with subsequent follow-up for 24 months.

KEY WORDS: glioblastoma, glioblastoma multiforme, cannabinoids, overall survival, prognosis.

2. ABBREVIATIONS

Δ-8-THC : Δ-8-tetrahidrocannabinol

2-AG: 2-Arachidonoylglycerol

AA: anaplastic astrocytoma

AEMPS: Agencia Española de Medicamentos y Productos Sanitarios

ALT: alanine aminotransferase

AO: anaplastic oligodendroglioma

AST: aspartate aminotransferase

BCP: beta-caryophyllene

cAMP: cyclic adenosine monophosphate

CB1: cannabinoid receptor type 1

CB2: cannabinoid receptor type 1

CBD: cannabidiol

CBN: cannabinol

CEIC: Clinical Research Ethics Committee

CNS: central nervous system

CRO: Contract Research Organization

CSF: cerebrospnial fluid

CT: chemotherapy

CTCAE: Common Terminology Criteria for Adverse Events

DD: dense dose

ECOG: Eastern Cooperative Oncology Group

EGFR: epidermal growth factor receptor

EGFRvIII: epidermal growth factor receptor variant III

ET-1: endothelin-1

FAAH: fatty acid amide hydrolase

FAK: focal adhesión kinase

FDA: Food and Drug Administration

GBM: glioblastoma multiforme

GSCs: glioma stem-like cells

HSP: heat shock proteins

ICO: Institut Català d'Oncología

IDH: isocitrate dehydrogenase

iGP: inhibitory G proteins

KPS: Karnofsky performance scale

LOH: loss of heterozygosity

MAGL: monoacylglycerol lipase

MAPK: mitogen-activated protein kinases

MGMT: O(6)-methylguanine-DNA methyltransferase

MMP2: matrix metalloproteinase-2

MMP9: matrix metalloproteinase-9

MMSE: mini mental state examination

MRI: magnetic resonance imaging

mTOR: mammalian target of rapamycin

OV: overall survival

PDGF-AA: platelet-derived growth factor-AA

PFS: progression-free survival

PI3K: phosphoinositide 3-kinases

PKC: protein kinase C

PTEN: phospatase and tensin homolog

RANO: Response Assessment in Neuro-Oncology

ROS: reactive oxidative species

RPA: recursive partitioning analysis

RT: radiotherapy

RTOG: Radiation Oncology Group

SEOM: Sociedad Española de Oncología Médica

THC: Δ-9 -tetrahidrocannabinol

TMZ: temozolomide

TTF: tumor treating fields

TTP: time to tumor progression

ULN: upper limit of normal

UPA: urokinase-type plasminogen activator

3. INTRODUCTION

3.1. Generalities

The central nervous system (CNS) tumors can be divided into two large groups: primary lesions, which originate from cells that belong to the CNS and are the primary tumors themselves; secondary lesions, which originate in other parts of the body and are implanted as metastases in the CNS.

Primary CNS tumors are a heterogeneous group of neoplasms according to the cell line from which they originate; the global incidence is 2.8-7.8 cases per 100.000 inhabitants/year, with a difference between sexes (3.9 men and 3.0 women per 100.000 inhabitants/year) (1) and secondary tumors of 1-3 cases per 100.000 inhabitants/year *(Table 1)*. However, if the data is analysed by histological types, brain metastases are the most frequent CNS tumors (9-16.2 cases per 100.000 inhabitants/year), followed by meningiomas (7.8-8 cases per 100.000 inhabitants/year) and glioblastoma (GBM) (2.4-3.3 cases per 100.000 inhabitants/year) (2). In Spain, the incidence is 8.73 in men and 5.41 cases per 100.000 inhabitants/year in women (3).

Most of the primary tumors are benign, up to 66%; however, many benign tumors are not histologically confirmed by the difficulty involved in biopsying such a sensitive organ, so the incidence of malignant tumors could be greater than 44% as described by some authors. On the other hand, it seems that the incidence of primary malignant lesions is slightly higher in males (55% vs. 45%) while benign primaries, such as meningiomas, occur more frequently in females (64% vs. 36%) (2).

During 2012, in Europe, there were approximately 57.100 cases of brain tumors and CNS *(Table 1)*, which represents 6.6% of the total cancers (4).

Table 1. Incidence, mortality and prevalence of brain and CNS cancer in 2012 (5).

EUROPE	**Incidence**	**Mortality**	**Prevalence**			
	Number	*Rate*	*Number*	*Rate*	*1 year*	*5 years*
Men	30.715	7.8%	24.554	6%	12.543	34.861
Women	26.284	5.6%	20.425	4%	10.008	27.630
Both	57.099	6.6%	44.979	4.9%	22.551	62.491
SPAIN	**Incidence**	**Mortality**	**Prevalence**			
	Number	*Rate*	*Number*	*Rate*	*1 year*	*5 years*
Men	2.056	7.7%	1.661	5.3%	683	1.898
Women	1.469	5.3%	1.199	3.5%	472	1.289
Both	3.717	6.5%	2.668	4.4%	1.155	3.187

The most frequent location of brain tumors are the meninges (36.2%) hence the highest incidence of meningiomas. Of the supratentorial intra-axial tumors, the most frequent location is the frontal lobe (8.6%) (2).

When comparing by age, the total incidence of brain tumors is higher in patients older than 85 years and lower in 0 to 14 years children. In patients under 15 years, the incidence is up to almost 15%. Pielocytic astrocytomas, germ cell tumors and embryonic tumors are more frequent in this age group and their incidence decreases in older groups, unlike GBM, whose incidence increases with age (6).

In relation to gender, in 2000 the mortality rate in Spain for the male population was 6 out of every 100.000 inhabitants (1.259 men), and in women 4 per 100.000 inhabitants (1.000 women) (7).

The 2016 World Health Organization (WHO) classification (ANNEX 1) classifies CNS tumors, as we have already said, according to the cell from which it originates. There are two large groups: the epithelial and non-epithelial origin. The first is formed by those that originate in astrocytes such as astrocytoma and GBM; those that come from oligodendrocytes such as oligodendroglioma; ependymoma if it comes from ependymal cells; neuroblastoma and medulloblastoma if they originate in the sympathetic nervous system or very undifferentiated cells respectively *(Figure 2)*. In the second group are the primary cerebral lymphoma and meningioma, among others. Both groups are again stratified according to histological grade: low (I and II) or high (III and IV).

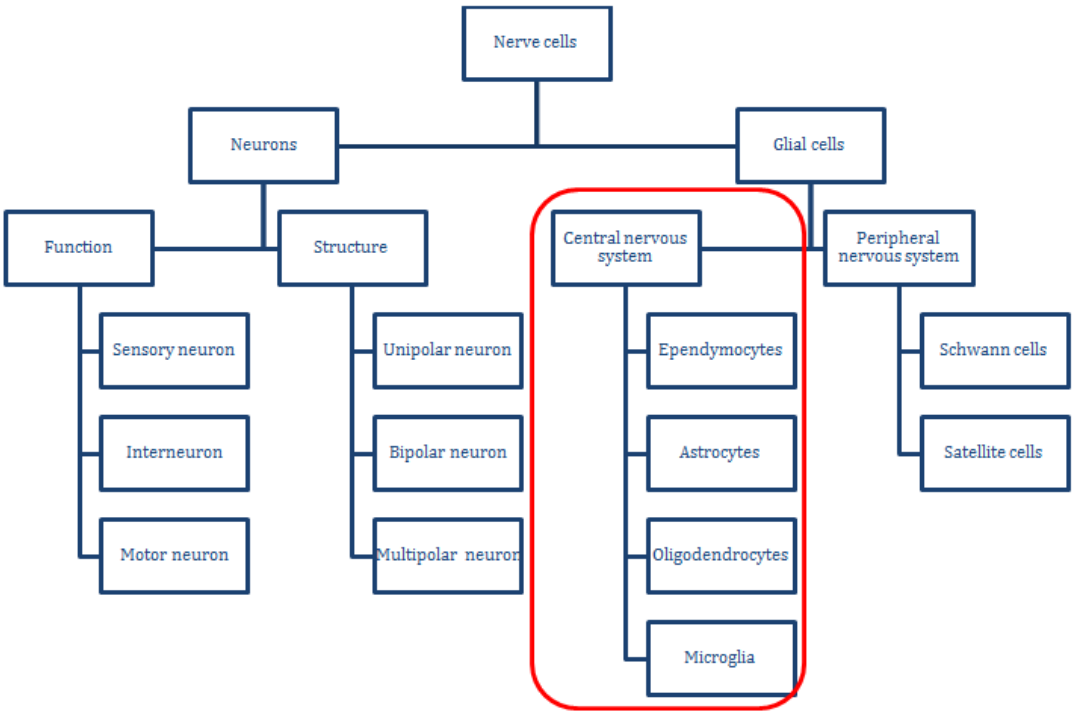


Figure 2. Surrounded in red all those cells from which tumors of the central nervous system are derived, such as GBM, which is the most frequent astrocytoma.

The degree relates to the prognosis and aggressiveness. Depending on the severity, CNS tumors are:

- Low grade (I and II): they have a low proliferative potential, are well differentiated and have a better prognosis for the patient.
- High grade (III and IV): they have more atypia and mitosis, are more undifferentiated and have a worse prognosis. Both have microvascular hyperplasia, but what differentiates grade IV is the presence of necrosis. The GBM is therefore placed as grade IV.

Another way to classify these tumors is according to their location (8,9): supratentorial or infratentorial *(Table 2)* depending on whether they are located above or below the tentorium (extension of the dura mater that separates the cerebellum from the lower portion of the occipital lobe of the brain).

Table 2. Classification according to the location in relation to the tentorium.

Location		
Supratentorial	Adults: 80-85%	Children: 40%
Infratentorial	Adults: 15-20%	Children: 60%

3.2. Glioblastoma multiforme

The most common type of astrocytoma is GBM. This is the most common primary malignant brain tumor in adults with a mean age at diagnosis of 64 years. Its 5-year survival is less than 5% (6,10,11). It represents 12-15% of all brain tumors, 50-70% of all astrocytic tumors and 80% of malignant astrocytomas (12).

The incidence in Girona, data from 1994 to 2013 of the Girona Cancer Registry, is 4.17 (95% CI 3.80-4.57) cases per 100.000 inhabitants/year, with a slight predominance in men. As for survival, in the first year it is 24% (95% CI 20.4-28.3), and 3.3% in 5 years (95% CI 2.0-5.6). It presents an overall survival (OS) of 24% at 1st year (95% CI 20.4-28.3), and of 3.3% at 5 years (95% CI 2.0-5.6). And a relative survival of 24% (95% CI 20.8-28.7) at first year and of 3.7% at 5 years (95% CI 2.3-6.2) (13)**.** Nevertheless, these dates have changed a bit since these data were collected before 2005, the year in which the current treatment of GBM was approved, as we will explain later.

Thus, GBM, as I said, is a supratentorial grade IV neuroepithelial tumor. It is characterized by its high cell proliferation and angiogenesis resulting in a rapidly growing tumor and, consequently, with necrosis. GBM cells also have high migration capacity and invasive properties, which allows them to produce metachronic lesions and spread through the brain parenchyma. In addition, the GBM contains a subpopulation of glioma stem-like cells (GSCs), which, at least partially, explains the treatment resistance and high recurrence rates.

It is located in the cerebral hemispheres although it can spread through the cerebrospinal fluid (CSF) to the spine very sporadically (14)**.** Direct access through the dura mater vessels to the extrameningeal tissue is considered the most likely route in the development of extraneural metastases, which some authors blame for surgical intervention (8)**.**

Most of them arise de novo, without evidence of a previous precursor injury, and are called primary GBMs. Secondary GBMs develop from lower grade astrocytomas (II or III) and are more prevalent in young patients (15). Although they are morphologically similar, these two groups have clinical characteristics and a different molecular profile *(Table 3)*. The first ones constitute 95% of the GBMs and have a natural history of less than 6 months, with an average incidence age of 64 years. Secondary GBMs represent only 5% of all GBMs and come from low grade tumors (II or III); they usually appear in younger patients with a median of 45 years (12,16).

Various genetic factors have been associated with its ethology, such as the overexpression of genes that control growth factors, specifically the amplification in the epidermal growth factor receptor (EGFR) in those primary GBMs (17), as well as in the platelet-derived growth factor receptor (PDGFR) that occur at 40 -57% and 60% respectively (15), two tyrosine kinases receptor of this big family of cell surface receptors. Other mutations target the gene of the mouse double minute homolog 2 (MDM2) in 10-15% and the phospatase and tensin homolog (PTEN) gene in 20-34% of cases (15).

Table 3. Differences between primary and secondary GBM [aa: anaplastic astrocytoma; ao: anaplastic oligodendroglioma; MGMT: O(6)-methylguanine-DNA methyltransferase; IDH: isocitrate dehydrogenase; LOH: loss of heterozygosity] (adapted from 15,18).

	PRIMARY GBM	**SECONDARY GBM**
Frequency	More frequent: >90%	Less frequent>10%
Evolution	Rapidly development (<3 months)	From <1 year to >10 years (Mean interval: 4-5 years)
Age	Older patients (62 years old)	Younger patients (45 years old)
Median survival time from diagnosis of GBM	4,7 months	7,8 months
Malignant precursor	Absent	Present: grade II-III astrocytoma
Genetic pathways (% of frequency)		
-MGMT promoter metilation	50%	75%
-IDH1/IDH2 mutations	<10%	70%
-EGFR amplification	35%	Rarely (8%)
-PTEN mutation	25%	Rarely (4%)
-TP53 mutation	30%	65%
-LOH 10q	50%	-
-LOH 10q	70%	63%
-LOH 19q	-	>50% (AA)
-Loss 1p/19q	-	>75% (AO)

The symptoms of GBM vary according to size, location and growth rate. Symptoms are slowly progressive unless the first manifestation is a seizure. The most common signs and symptoms include: headache, nausea or vomiting, confusion, cognitive impairment, memory loss, personality changes or behavioural disturbances, balance problems, motor disturbances, urinary incontinence, visual problems, speech difficulties and seizures.

The diagnosis of GBM is given after the pathological study. After the maximum possible surgical resection of the mass observed by brain magnetic resonance imaging (MRI), it is sent to the pathology laboratory in which the expert neuroanatomopathologist will study the sample and confirm the existence of cancer cells. In addition, the molecular characteristics that provide prognostic information will be analysed, further explained in the “prognostic” section.

3.2.1. Treatment

The standard treatment of gliomas has been until a few years ago the tumor resection and radiotherapy (RT), although in the last decade chemotherapy (CT) has become an established treatment in most histological subtypes.

Despite the treatment, the GBM always relapses. Since 2005, standard GBM therapy has not changed. Stupp, R., et al., demonstrated that the best treatment is *(Figure 3)*: maximum possible surgical resection, followed by a treatment based on CT (temozolomide, TMZ) at a dose of 75 mg/m2 daily, seven days a week from the first day until the last day of the concomitant RT, with daily focal RT at a total dose of 60 Gy (2 Gy/day) five days a week (Monday through Friday) for 6 weeks. After a 28-day break it is restarted with adjuvant TMZ 150-200 mg/m2 for 5 days every 28 days, until 6 cycles are completed (19).

As we will explain later, this study meant an increase in survival, when compared to the group receiving exclusive local focal RT after surgery. With a median follow-up of 28 months, the mean survival was 14.6 months (RT and TMZ) and 12.1 months with RT alone (HR 0.63; 95% CI 0.52-0.75; p <0.001). The two-year survival rate was 26.5% in the RT plus TMZ group and 10.4% with RT alone.

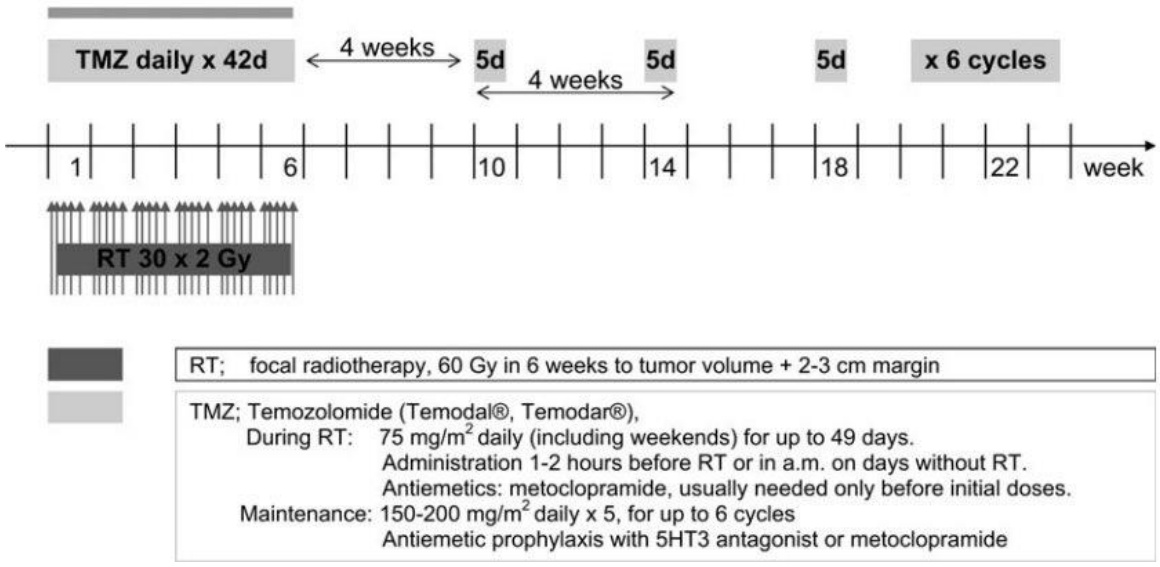


Figure 3. Current GBM treatment scheme GBM proposed by Stupp, R., et al. (19).

In addition, these patients need symptomatic treatment that may remain long periods of time:

- Corticosteroids: reduce edema but does not prevent tumor growth. If a symptomatic event requires them, an intravenous loading dose will be given. The treatment will be maintained and reduced gradually with time.
- Antiepileptic: the antiepileptic that has fewer interactions with chemotherapeutics is levetiracetam. Post-surgery will be given prophylactically post-surgery for a week and then withdrawn or kept in case there are seizures.
- Anticoagulants: in case of pulmonary thromboembolism or deep venous thrombosis. They occur in 20% of patients because damage to the brain parenchyma causes thromboplastin release (20).

Since 2005, several clinical trials have been conducted that attempt to improve outcomes for patients with GBM. For example, the *Radiation Therapy Oncology Group* (RTOG, Philadelphia, PA, USA) *0525* was a phase III trial that compared conventional adjuvant TMZ with TMZ at dense dose (dd). Despite confirming the prognostic importance of the MGMT promoter methylation, survival did not improve with dd TMZ (21). The addition of bevacizumab, an antiangiogenic in the study of the Radiation RTOG 0825 demonstrated an improvement in progression-free survival (PFS), however, it did not produce changes in OS (22). The addition of everolimus, an oral inhibitor of the mammalian target of rapamycin (mTOR), to chemoradiation, increased toxicity but had no impact on PFS survival and even shortened OS (23).

In 2011, the first-generation tumor treatment device (tumor treatment fields or TTF), currently known as the NovoTTF-100A System (called Optune), was approved by the *Food and Drug Administration* (FDA) for the treatment of recurrent GBM or refractory. More recently, in 2015, the FDA approved the TTF device as an adjuvant treatment for newly diagnosed patients after completing standard surgery and chemoradiation by demonstrating a better PFS [7.1 months on the TTF device plus the TMZ group and 4.0 in the TMZ group alone (p = 0.001)] (24).

The TTF device includes four sets of transducers, each consisting of nine insulated electrodes that are applied to the patient's shaved scalp to supply alternate low-intensity electric fields with an intermediate frequency (100–300 kHz) (24,25), operating with a battery pack. The device weighs approximately 5 kg.

In addition to this progress, additional tests are being carried out to analyse the use of immune control point inhibitors, such as Ipilimumab and Nivolumab (NRG-BN002). Also, a phase II trial of neoadjuvant TMZ followed by accelerated hypofractionated RT (60 Gy in 20 fractions) demonstrated a median of 22-month OS with a 13.2-month PFS, which compares favourably with the previously reported OS in other clinical trials (26).

Despite all these advances, research and studies, the prognosis of the GBM is austere. Therefore, the objective of this phase II clinical study that we present is to improve the prognosis, for it to be feasible and without a high economic impact, as well as without deteriorating the quality of life of patients.

3.2.2. Prognostic

Surgical resection degree will determine, in part, the patient's survival. It has a fundamental role; it has been seen that leaving a residual volume less than 5 cm3 (70%) improves the prognosis. A total resection leads to a survival of 20 months vs. 13 months if the resection is partial (27).

Other important prognostic factors are molecular alterations such as methylation of IDH and MGMT.

- Mutated IDH (usually occurs in young patients <32 years) vs. IDH *wild type* (more frequent in> 59 years) have a survival of 32 vs. 15 months respectively (28,29) . The IDH, 1 & 2, are involved in blocking cell differentiation, angiogenesis and epigenetic remodeling. The IDH2 gene is located in mitochondria and IDH1 in the cytoplasm. Due to the mutation of these genes an increase of 2-HG is detected, which causes an

accumulation of HIF-alpha and with it an increase in angiogenesis, as well as the inhibition of histone demethylases and the reduction of 5-hmC would produce aberrant DNA hypermethylation and chromatin remodelling (30,31).

- MGMT methylation is a predictor of TMZ response as well as a prognostic factor associated with greater survival, 18 vs. 12 months (28). The methylation of MGMT does not eliminate the methionine group that TMZ adds to the tumor DNA and therefore improves the response.

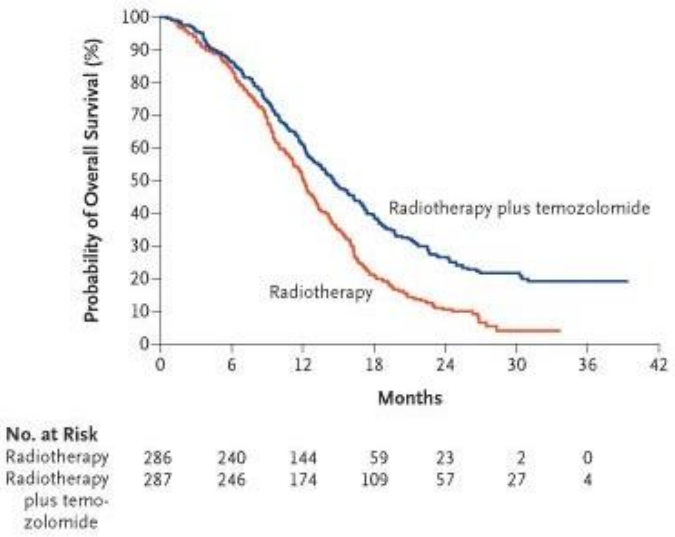
Other prognostic factors (27,32,33) are:

- Patient's age. An age <65 years is associated with a better prognosis.
- Neurological status of the patient and Karnofsky index (KPS). A KPS greater than 70 is associated with a better prognosis.
- Tumor location: this is related to the degree of resection and the patient's subsequent neurological deficit. Therefore, the periventricular location and tumors that cross the midline are of poor prognosis.
- Proliferative index (high Ki 67).
- Genetic alterations of the tumor, as in the epidermal growth factor receptor variant III (EGFRvIII).
- Not combining RT with TMZ is associated with a worse prognosis and, as we will see below, survival decreases.
- Postoperative complications, as well as post-intervention cognitive impairment, are associated with a worse prognosis.
- The possibility of a surgical reintervention in relapse is a factor of good prognosis.

If we individualize each patient, the combination of these parameters helps to estimate the prognosis (27,34). Other authors have pointed out as possible predictors of worse OS: asthenia and depression (35).

There are some prognostic classifications, such as the *recursive partitioning analysis* (RPA) described by the RTOG group and validated by *Eastern Cooperative Oncology Group* (ECOG) that is used because it has demonstrated a correlation with the benefit of treatment in terms of survival. It is longer for patients with favourable characteristics (RPA III-IV).

Despite treatment, survival is still very low: 14.6 months on average (95% CI 13.2-16.8) with the standard R. Stupp treatment, and 12.1 months (95% CI, 11.2-13.0) with only RT *(Figure 4-5)*. 100% of patients will develop a short-term recurrence, with a mean PFS of 6.9 months (95% CI, 5.8-8.2) in those receiving this scheme and 5 months (95% CI, 4.2-5.5)for those who only receive RT (19).



Variable	Radiotherapy (N=286)	Radiotherapy plus Temozolomide (N=287)
	value (95% CI)	
Median overall survival (mo)	12.1 (11.2–13.0)	14.6 (13.2–16.8)
Overall survival (%)		
At 6 months	84.2 (80.0–88.5)	86.3 (82.3–90.3)
At 12 months	50.6 (44.7–56.4)	61.1 (55.4–66.7)
At 18 months	20.9 (16.2–26.6)	39.4 (33.8–45.1)
At 24 months	10.4 (6.8–14.1)	26.5 (21.2–31.7)
Median progression-free survival (mo)	5.0 (4.2–5.5)	6.9 (5.8–8.2)
Progression-free survival (%)		
At 6 months	36.4 (30.8–41.9)	53.9 (48.1–59.6)
At 12 months	9.1 (5.8–12.4)	26.9 (21.8–32.1)
At 18 months	3.9 (1.6–6.1)	18.4 (13.9–22.9)
At 24 months	1.5 (0.1–3.0)	10.7 (7.0–14.3)

Figure 4 and 5. Comparison of survival data and progression intervals in the subjects of the Stupp study (19).

Since recurrence is the inevitable evolution of GBM, radiological follow-up is necessary every 2-3 months with cranial MRI, although treatment after relapse is limited and palliative in most cases (36). Bevacizumab with or without irinotecan, fotemustine or TMZ have proven beneficial as a second-line treatment in GBM recurrences.

In conclusion, the prognosis of the GBM is unfortunate, the treatment remains the same as 14 years ago and there are no known advances, due to economic problems (e.g. NovoTTF) or the lack of scientific evidence.

Being the PFS of almost 7 months, the objective of this TFG is not to assess the time to progression in 6 months, since most do not progress at 6 months, but to assess the OS at 12 months. This survival is currently 61% *(Figure 4 and 5)* (19), and with concomitant treatment with cannabinoids we expect it to be 80% at 12 months.

The objective of this TFG is the realization of a protocol that supposes an alternative in patients with GBM and that obtains a greater percentage of one-year OS: the cannabinoids.

3.3. Cannabinoids

Cannabinoids are chemical substances that can be external, taken from *Cannabis sativa* or synthetic, or internal, and that bind to specific receptor proteins within the body such as cannabinoid receptor type 1 (CB1) and type 2 (CB2), acting as agonists or inverse agonists. Endocannabinoids are distributed throughout the body and have a broad spectrum of action on neuro-immuno-endocrine activity, so they induce similar effects to those produced by the *Cannabis sativa* plant.

Therefore, three types of cannabinoids are known (37):

- **Phytocannabinoids:** naturally synthesized by the *Cannabis sativa* plant. The main phytocannabinoids are Δ-9-tetrahydrocannabinol (THC), Δ-8-tetrahydrocannabinol (Δ-8-THC), cannabidiol (CBD), beta-caryophyllene (BCP) and cannabinol (CBN). THC is primarily responsible for the psychoactive effects of this plant.
- **Endogenous cannabinoids or endocannabinoids:** produced by the human body and in animals. Some of them are: anandamine (arachidonic acid amide) and 2-arachidonyl glycerol (2-AG) synthesized from cell membrane phospholipids. After exerting their biological function, they are degraded by a fatty acid amide hydrolase (FAAH) and by monoacrylic glycerol lipase (MAGL), which converts these endocannabinoids into arachidonic acid and glycerol, respectively. In GBM, increased levels of anandamide and reduced activity of the fatty acid amide hydrolase degrading enzyme (FAAH) have been identified (38).

  Endocannabinoids are synthesized, released, recaptured and degraded in the nerve cells of the hippocampus, thalamus, striatum, cerebral cortex, brainstem, cerebellum and spinal cord. They produce pharmacological effects similar to those of THC but with a much shorter duration of action. In addition, unlike other neurotransmitters, they are not synthesized in advance or stored in presynaptic vesicles.
- **Synthetic cannabinoids:** similar compounds that are generated in the laboratory.

Cannabinoids with therapeutic interest are those that are absent from the effects of intoxication: CBD and BCP (39,40).

CBD has activity as an inverse agonist of CB2, it behaves as a high potency antagonist of cannabinoid receptor agonists in brain tissue (41–43).

On the other hand, CBD targets several G-protein coupled receptors and also TRPV1 and TRPV2 vanilloid receptors (non-selective cationic channels involved in pain transmission and modulation) (44–46).

We differentiate two types of cannabinoid receptors (37):

- **CB1**: found mainly in the CNS, with higher density at the basal ganglia, cerebellar molecular layer, cerebral cortex and in lower density in certain parts of the hippocampus. In addition, they are also found in the reproductive systems (prostate, uterus and ovary), digestive and immune (spleen and tonsils).

  The distribution of these is closely related to the pharmacological effects that cannabinoids produce. Thus, the high density of receptors in the basal ganglia is related to the marked effects that these compounds exert on the locomotive activity, leading to produce catalepsy at high doses. The presence in hippocampal and cortical areas would explain the effects of cannabinoids on learning and memory, as well as their anticonvulsant properties. Finally, the low density of receptors in the brainstem explains the low toxicity and absence of lethality of marijuana.
- **CB2**: found in peripheral tissues such as tonsils, spleen and testicles, as well as in immune system cells such as monocytes, macrophages, B lymphocytes and T lymphocytes. These appear to be responsible for the immunosuppressive properties of marijuana.

Both belong to the family of G-protein coupled receptors characterized by the presence of seven transmembrane domains.

There is a general consensus that high-grade gliomas, including GBM, express high levels of CB2. In addition, the expression of CB2 correlates positively with the degree of malignancy (47). On the contrary, the expression of CB1 still requires characterization, since it has been reported that it has not changed (48), decreased (49) or even increased (50,51) in GBM compared to low-grade gliomas or tissues without tumor control.

The activation of cannabinoid receptors inhibits adenylate cyclase, with the consequent decrease in the intracellular concentration of cyclic adenosine monophosphate (cAMP), ceramide signals and induces phosphorylation of focal adhesion kinase (FAK), of mitogenated activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K). They also regulate the expression of immediate early genes and regulate the production of nitric oxide (41). In

addition, being associated with inhibitory G proteins (GPi), when activated, a blockage of calcium entry into the cells and an increase in potassium conductance occurs; the combined effect on both types of channels is the basis of inhibition that cannabinoids exert in the release of neurotransmitters (52).

In this way, the phosphorylation capacity of protein kinases dependent on this cyclic nucleotide is affected, which given the functions that these kinases exert on cell metabolism or genetic expression will lead to certain biological effects *(Figure 6).*

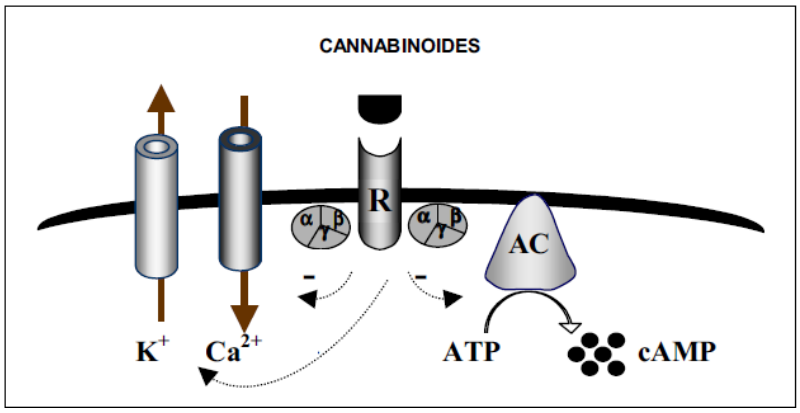


Figure 6. Changes in cannabinoid receptors (37).

3.3.1. Cannabinoid functions

Exogenous cannabinoids produce effects on the activity of neurotransmitters in the brain, both at the level of dopamine and serotonin, γ-aminobutyric acid GABA, glutamate and opioid peptides.

Endocanabinoids have been implicated in multiple biological processes. Based on their location and mechanism of action, as well as the interaction with neurotransmitters, the stimulation of cannabinoid receptors, whether external or internal, can produce the following responses:

- Feeling of euphoria, sedation and relaxation
- Changes in temporal perception (overestimation of elapsed time) and recent memory
- Analgesic and anti-inflammatory activity
- Orexigenic and antiemetic activity
- Actions on muscle tone and motor coordination (ataxia, muscle weakness)

- Decrease in intraocular pressure
- Hypothermia
- Actions on the respiratory system (bronchodilation)
- Cardiovascular effects (hypotension and tachycardia)
- Neuroendocrine effects (decreased release of different sex hormones, and increases in the release of hormones related to the stress response)
- Immunomodulatory effects (low dose immunostimulation and high dose immunosuppression)
- Antiproliferative and antitumor effects

With this, the possible therapeutic use of cannabinoids is evident. Thus, J. J. Meana and I. Ulibarri, 1999, proposed the main pharmacological actions of cannabinoids (37) that could produce a benefit:

- Contraception
- Analgesia
- Antiemesis
- Anticonvulsants
- Angiogenesis and psychotomymesis
- Oxygen activity
- Hormonal and immune control

Currently in the Spanish state, the only therapeutic indication of cannabinoids is the treatment for the improvement of symptoms in adult patients with moderate or severe spasticity due to multiple sclerosis who have not responded adequately to other medically antispastic and who have shown a clinically significant improvement of symptoms related to spasticity during an initial trial period of treatment. This drug is Sativex®, a solution for oral spraying that contains 2.7 mg of THC and 2.5 mg of CBD. This medication, as we will explain in detail later, will be the one administered in our phase II clinical trial.

3.3.2. Effect of cannabinoids on glioblastomas

Several investigations have revealed the neuroprotective and glycoprotective effects of cannabinoids, through a decrease in neurotoxicity, such as secondary to glutamatergic overexcitation, in addition to its own antioxidant nature. Several studies have demonstrated the efficacy of cannabinoids in GBM (53,54).

The best studied effect of cannabinoids on GBM pathophysiology is tumor growth inhibition. Diverse in vivo studies showed that cannabinoids could significantly reduce tumor volume in orthotopic and subcutaneous glioma animal models (55). The mechanisms that mediate this phenomenon can be grouped into three categories:

1. Cell death inducing mechanisms (apoptosis and cytotoxic autophagy)
2. Anti-angiogenic mechanisms
3. Cell proliferation inhibitor mechanisms

3.3.3. Cell death: apoptosis and cytotoxic autophagy

THC and CBD, both natural and synthetic, induce cell death by apoptosis of transformed cells of glial and neuronal origin. Also, this effect does not imply a mere generalized toxic action, since it is quite selective of tumor cells. Unlike what happens in the tumor cells of the glia, the viability of astrocytes in culture is not affected by cannabinoids (56). This differential behaviour of transformed and non-transformed glial cells could reside in the ability of the first ones but not the second to synthesize de novo a bioactive sphingolipic, ceramide, in response to cannabinoids (53,57). Accordingly, astrocytes suffer apoptosis when de novo synthesis of ceramide is selectively induced in these cells by certain drugs. It has been observed that cannabinoids rescue astrocytes in culture by ceramide-induced apoptosis, an effect that depends on the activation of cannabinoid receptors (CB1).

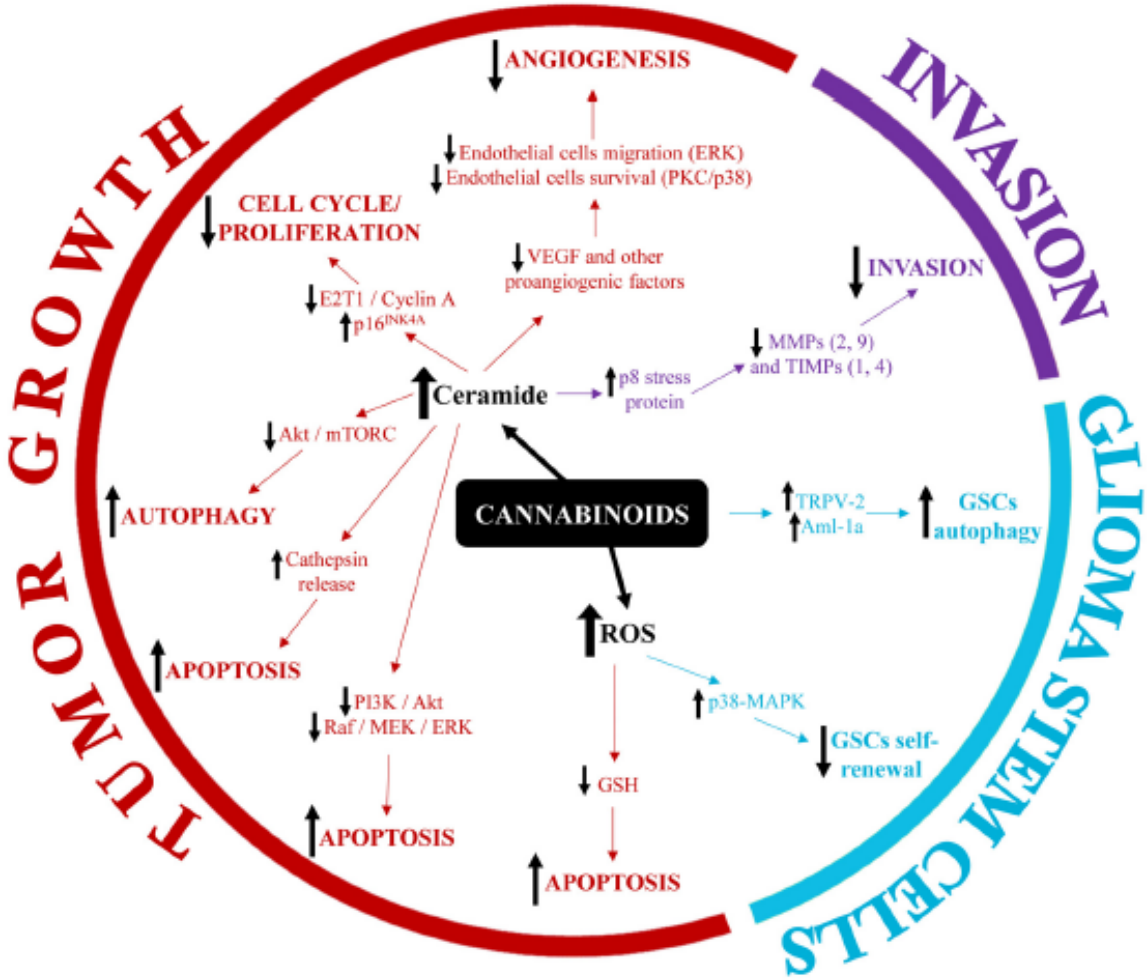


Figure 7. Molecular mechanisms of action of cannabinoids (57).

Cannabinoid-induced cell death occurs primarily through the intrinsic (mitochondrial-dependent) apoptotic pathway mediated by an increase in intracellular ceramide, which inhibits the survival pathways pro PI3K/Akt and Raf1/MEK/ERK *(Figure 7)* allowing pro-apoptotic Bcl-2 Bad to phosphorylates and translocates to mitochondria (47). This results in the loss of integrity of the external mitochondrial membrane, the release of cytochrome c and the activation of apoptosis executing caspases.

Interestingly, ceramide has also been implicated in cannabinoid-induced autophagy of glioma cells through the p8/TRB3 pathway and subsequent inhibition of the Akt/mTORC1 axis (58,59). Recent studies also showed that THC altered the balance between ceramides and dihydroceramides in autophagosomes and autolysosomes, which promoted the permeabilization of the organelle membrane, the release of cathepsins in the cytoplasm and the subsequent activation of apoptotic cell death (60).

In addition to ceramide-mediated cell death, cannabinoids were also shown to trigger apoptosis through oxidative stress and formation of reactive oxygen species (ROS) (61,62). Specifically, CBD-treated glioma cells responded with production of ROS, depletion of GSH and activation of caspase-9, -8 and -3. However, Scott, K., et al. showed in 2015 that while CBD treatment of glioma cells induced a significant increase in ROS production, this phenomenon was accompanied by a positive regulation of a large number of genes belonging to the superfamily of the protein of thermal shock (HSP). Therefore, it has been proposed that the inclusion of HSP inhibitors could improve the antitumor effects of cannabinoids in GBM treatment regimens (63).

3.3.4. Anti-angiogenic mechanisms

Besides the mechanisms already mentioned, it has been seen that in vivo the situation is more complex. Specifically, it has been seen that cannabinoids appear to block tumor growth by also inhibiting angiogenesis.

In preclinical studies, the local administration of cannabinoids to mice inhibited the angiogenesis of malignant gliomas (64). Furthermore, the local administration of THC decreases the levels of pro-angiogenic VEGF in recurrent GBM (64). In vitro, cannabinoids inhibited endothelial cell migration through the ERK pathway and endothelial cell survival through protein kinase C (PKC) pathways (65); CBD induces cytostasis of endothelial cells, inhibits migration and growth of endothelial cells in vitro and inhibits angiogenesis, accompanied by down regulation of pro-angiogenic factors such as metalloprotease-2 and -9 matrix (MMP2 and MMP9), urokinase type plasminogen activator (uPA), endothelin-1 (ET-1), platelet-derived growth factor-AA (PDGF-AA) and chemokine ligand 16 (66).

It has been studied whether the antitumor effect of cannabinoids could be due to the modulation of the immune system: in studies with immunocompromised rodents a decrease in tumor size has been seen in regard to animals of the control group. This indicates that the antiproliferative effect of cannabinoids is exerted directly on the tumor (67).

3.3.5. Mechanisms inhibiting cell proliferation

Additionally to a direct destructive effect on tumor cells and an inhibition of angiogenesis, cannabinoids can also induce cell cycle arrest, thereby inhibiting the proliferation of tumor cells.

For instance, the treatment of GBM cells with THC and/or CBD increases the population of cells in the G0-G1 phase and the G2-GM phase while decreasing the number of cells in the S phase (62). Similarly, administration of THC to human GBM cell lines induces the arrest of the G0-G1 phase; THC also lowers the levels of E2F1 and Cyclin A (two proteins that promote cell cycle progression) while regulating the level of the cell cycle inhibitor p16INK4A (68).

3.3.6. Anti-invasive effect

Although GBM rarely metastasizes, its tumor cells are capable of infiltrating the surrounding healthy brain tissue and spreading through the cerebral parenchyma as we have previously commented (69). Therefore, therapeutic strategies that are aimed to inhibit migration and invasion have clinical relevance in the treatment of this cancer.

Although its role is still poorly defined, the accumulated evidence suggests that cannabinoids have potent anti-invasive effects on glioma cells both in vitro and in vivo, even at low concentrations (70). For example, in 2013 Soroceanu, L. et al. showed through organotypic sections of the brain that CBD inhibited the invasion of GBM cells. This anti-invasive effect was attributed to the inhibition of DNA-binding protein inhibitor ID-1 expression by CBD and was observed in several GBM cell lines (71). In addition, CBD treatment significantly decreases metalloproteinases, major proteins associated with tumor invasion, in particular MMP-9 and TIMP-4 (70). This effect also occurs when THC is administered. These effects were mediated by the accumulation of ceramide and the activation of the stress protein p8 (58,72).

3.3.7. Psychoactive effects

It is desirable that therapies based on cannabinoids lack of psychoactive side effects.

Studies by the *Sociedad Española de Investigación sobre Cannabinoides* (SEIC) have shown that the CB2 receptor is abundantly expressed in gliomas, and that its selective activation with certain synthetic cannabinoids leads to the regression of these tumors in mice without psychoactive side effects.

Galve-Roperh et al. examined in 2000 the possible side effects of cannabinoid treatment. Thorough analysis by brain MRI of all animals whose tumors were eradicated with cannabinoids revealed that treatment with cannabinoids did not produced signs of damage due to necrosis, edema, infection, inflammation or trauma. To rule out the possibility that

cannabinoids were toxic to non-tumor nerve cells, specific histological stains were performed and demonstrated that these compounds did not produce any generalized apoptotic effect in the brain, but only in transformed cells. Likewise, in the blood tests the biochemical parameters and markers of tissue damage were not affected during the period of 7 days of administration or up to 2 months after the end of the cannabinoid treatment.

In addition, it has been found that cannabinoids can stop the growth of tumor cells in breast and prostate cancer (73) and induce in vivo the regression of lung adenocarcinomas (74), and thyroid epitheliomas (75).

4. JUSTIFICATION

GBM is the most common primary malignant CNS tumor in adults with a median survival of 14.6 months (95% CI 13.2-16.8) with the standard Stupp treatment (19).

It was in 2005 when Stupp R. et al showed that survival increased by 10% per year and 15% at two years if TMZ was administered concomitantly with RT after surgery and, subsequently, TMZ adjuvant monotherapy. Nowadays, the treatment of GBM has not changed: surgery, with maximum resection possible, and subsequent treatment with TMZ 75 mg/m2 concomitant with daily focal RT at a total dose of 60 Gy (2 Gy/day) for 6 weeks, 28-day rest and adjuvant TMZ 150-200 mg/m2 x 5 days every 28 days to complete 6 cycles (19).

Despite treatment, the majority of patients progress to a short-term recurrence, with a mean progression-free survival (PFS) of 6.9 months (95% CI, 5.8-8.2) in those receiving this scheme and 5 months (95% CI, 4.2-5.5) those who only receive RT (19), and a recurrence rate of almost 100% of patients regardless of treatment (19).

At one year, OS is 61% (95% CI, 55.4-66.7), at two years it is 26.5% (95% CI, 21.2-31.7) (19) and after five years it is less than 5% (10,11).

Due to the low survival rate of GBM and being considered an “orphan treatment” disease, investigations of new drugs and/or the approval of new indications for existing drugs are necessary.

Preclinical studies have shown that GBMs have type 1 and type 2 cannabinoid receptors, with more proportion of type 2 (47). Also, increased levels of anandamide and reduced activity of FAAH have been identified (38).

It was more than a decade ago that the effects of cannabinoids, specifically THC, were studied in patients with recurrent GBM (53). An increase in survival and time to progression was demonstrated.

Several in vivo preclinical studies showed that cannabinoids have antitumor effects, reducing tumor size in animal models. This effect is given by three processes:

1. Mechanisms that induce cell death (apoptosis and cytotoxic autophagy).
2. Anti-angiogenic mechanisms.
3. Mechanisms inhibiting cell proliferation.

In animal models of gliomas, cannabinoids inhibited growth (53,64) and angiogenesis. In addition, this antiproliferative effect appears to be selective for cancer brain cells and does not affect normal astrocytes (56,76).

Currently, there is no published any phase II clinical trial. However, in 2017 a press release was published in which it was reported that GW Pharmaceuticals found positive results in a phase II clinical trial (clinical trial NCT01812603) in which the standard treatment plus placebo was compared with the administration of THC and CBD in the standard treatment. The study showed that patients with recurrent GBM treated with THC:CBD had an overall survival of 83% per year compared to 53% of patients in the placebo cohort.

That is why my objective is the delivery of this protocol of a phase II clinical trial in which the one-year OS rate of newly diagnosed GBMs is evaluated by adding cannabinoids (Sativex®: THC and CBD) to their standard Stupp regimen, compared to those who will only receive the standard treatment plus placebo. This study would imply an important change in the prognosis of GBM, being something novel and interesting since there is no other published phase II clinical trial comparing the current treatment with and without cannabinoids.

Ethically, the principles of biomedical and human research will be respected: principle of non-maleficence and respect for people, beneficence, patient autonomy and justice.

5. HYPOTHESIS

Concomitant cannabinoids treatment with standard treatment of newly diagnosed GBM (Stupp scheme) improves one-year OS compared to those patients who only receive the Stupp regimen.

6. OBJECTIVE

The primary objective of this study is to determine whether adding cannabinoids to the base treatment of newly diagnosed GBM increases one-year OS rate with an optimal result of 80%, compared to those patients who only receive the standard treatment whose OS at one year is 61%.

We will randomize patients in two groups: group A, treatment group, patients will be treated with surgery, RT and CT in the classic scheme plus concomitant cannabinoids during CT and RT until progression; group B, control group, patients will receive the same Stupp scheme plus concomitant placebo treatment until progression.

As secondary objectives, we will assess the time to progression (TTP) and the treatment tolerance.

7. METHODOLOGY

7.1. Study design

Due to the pathophysiology of this disease, prolonged follow-up is necessary to properly assess the onset of disease progression.

The best way to confirm or reject our hypothesis, according to existing data and the limitations of this topic, would be a multicenter, longitudinal, prospective, double-blind, randomized and controlled clinical trial.

The study will be carried out in 3 hospitals of the Institut Català d'Oncologia (ICO) of the community: ICO of L'Hospitalet de Llobregat, ICO Badalona and ICO Girona. The ICO Girona, which works together with the Dr. Josep Trueta University Hospital, will be the reference center.

In each of the centers we will assign a principal researcher (a neurooncologist) who will propose to the patients to enter into the study and will follow them until progression, a neuroradiologist for the evaluation of brain MRI, a neurosurgeon and a reference neuropathologist who will perform and analyse the brain tissue samples.

The recruitment of patients will last 12 months, with subsequent follow-up of them for 24 months, in order to determine the OS per year and assess the time to progression in 24 months. In total, the study time is 36 months. Subsequently, the possible losses of the patients in the study and the end of it are explained.

7.2. Study subjects

To know which patients will participate in this study, we have to define our population of interest. The study subjects will be the newly diagnosed patients of GBM in the 3 ICO hospitals mentioned before and that meet the inclusion criteria and none of the exclusion criteria.

The patients will be selected and included in the study on the first day come to the oncologist consult after surgery or biopsy in the first 6 weeks during a recruitment period of one year.

7.2.1. Inclusion criteria

Patients may be included in the study only if they meet all of the following inclusion criteria at the time of patient selection:

- Ability to understand and the willingness to sign a written informed consent document.
- Patient ≥18 years old-70.
- Patients candidates for Stupp treatment with ECOG ≤ 2.
- Newly diagnosed GBM (WHO grade IV) histologically confirmed by biopsy or resection no more than 4 to 6 weeks before registration, including the following recognized variants of GBM: small cell GBM, giant cell GBM, gliosarcoma and GBM with oligodendroglial component.
    - Patients must have at least 15 unstained slides or 1 tissue block (frozen or paraffin embedded) available from a prior biopsy or surgery (archival tumor material).
    - Patients must have sufficient time for recovery from prior surgery (at least 4 weeks) and no more than 6 weeks from surgery.
- Laboratory test (≤ 2 weeks before the participation to the study):
    - Adequate hematologic function: hemoglobin ≥10 g/dl, Leukocytes > 3,000/mcL, neutrophils ≥ 1,500 cells/ul and platelets ≥ 100,000 cells/ul.
    - Adequate liver function: Bilirubin ≤ 2 time the upper limit of normal (ULN); Alkaline phosphatase ≤ 2,5; Aspartate aminotransferase (AST) ≤ 2.5 X ULN; Alanine aminotransferase (ALT) ≤ 2.5 X ULN. Except when attributable to antiepileptics or transient elevation in postoperative attributable to narcotics.
    - Creatinine within normal institutional limits (≤ 1,5) or creatinine clearance > 60 mL/min for subjects with creatinine levels above institutional normal.
- In the case of women: if they are of childbearing age, not be pregnant at the beginning of the study and accept contraceptive treatment to avoid possible pregnancy during treatment. In addition, the contraceptive should be potent and it is recommended double contraception, since Sativex® decreases the effectiveness of oral contraceptives; be in non-reproductive phase (i.e., post-menopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; history of hysterectomy, or history of bilateral tubal ligation, or history of bilateral oophorectomy).

7.2.2. Exclusion criteria

Patients will be excluded from the study for any of the following reasons:

- Presence of extracranial metastatic disease.
- Participants may not be receiving any other investigational agents.
- Hypersensitivity to cannabinoids, anhydrous ethanol, propylene glycol or peppermint essence.
- Patients must not have received prior cannabinoids treatment or have a background of substance abuse.
- Any surgery (not including minor diagnostic procedures such as lymph node biopsy) within 2 weeks of baseline disease assessments; or not fully recovered from any side effects of previous procedures.
- Any psychiatric or cognitive disorder that would limit the compromise compliance with the requirements of this protocol.
- Any clinically significant oral abnormalities, which may impair intake, transit or absorption of the study drug.
- Current (or within 6 months) significant cardiovascular disease including, but not limited to, myocardial infarction, cerebrovascular accident, transient ischemic attack or symptomatic pulmonary embolism, bradycardia defined as <50 bpm.
- Active and clinically significant infections.
- Patient with liver disorders (including but not limited to Gilbert's syndrome).
- Current use or anticipated requirement for drugs known to be moderate or strong cytochrome p450 inhibitors and strong or moderate cytochrome p450 3A4 inducers (coumarins, statins, beta blockers, rifampicin, fluconazole and ketoconazole).
- Individuals with a history of a different malignancy are ineligible except for the following circumstances: individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years and are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers are eligible if diagnosed and treated within the past 3 years: cervical cancer in situ, and basal cell or squamous cell carcinoma of the skin. Patients will not be eligible if they have evidence of other malignancy requiring therapy other than surgery within the last 3 years.

- Patients who have had prior stereotactic RT, convection enhanced delivery or brachytherapy (as gliosis/scarring from these modalities may limit delivery).
- Patients present with leptomeningeal dissemination.
- HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions. In addition, these individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy.
- Other severe acute or chronic medical condition, uncontrolled intercurrent illness or laboratory abnormality that may increase the risk associated with trial participation or investigational product administration or may interfere with the interpretation of trial results and, in the judgment of the investigator, would make the patient inappropriate for entry into this trial.
- Known or suspected personal history or family history of schizophrenia or other psychotic, a history of serious diseases important personality disorder or other psychiatric disorders other than depression associated with the underlying disease.
- Women breast feeding.

7.2.3. Withdrawal and replacement of patients

Every effort should be made within the bounds of safety and patient choice to have each patient complete the study. Patients who start the follow-up should continue to be followed for progression-free and OS as per protocol, unless there is a justified motive. Reasons for patient removal from the study follow-up include:

- Request of the patient or the patient's legal representative (withdrawal of consent for the study follow-up).
- Patient lost to follow-up. A patient should be considered lost to follow up only after de multiple efforts have been made to contact the patient to assess his/her health status and after de failure of the patient to attend scheduled visits. If after the two documented phone calls the investigative is still unable to contact the patient, he or she will be considered lost to follow up.
- Patients with deterioration that disables them to come to the consultation and/or take the treatment.

A record of the patient being lost to follow up should be noted with their documents along with the reason and the phone contacts.

As study includes patients by to consecutive sampling, replacement of patients is possible during the period of recruitment in order to have enough people.

7.2.4. End of the study

All patients will be followed with regard to survival until death, discontinuation from study follow-up, or termination/completion of study. Patients who die or complete the study follow-up through study closure will be considered to have "completed" the study.

The clinical trial will be considered complete when sufficient data is obtained to conclude the study (as defined in the statistical plans). It is estimated that patients may be followed during two years. In total, the trial has 12 months of recruitment and 24 months of follow-up. The study will end after the statistical analysis.

The evaluation of patients will be finished if patients are able to go to the consultation visits until the end of treatment. This follow-up will be stopped if death or tumour progression occurs (defined in section "Study Variables").

7.3. Sample selection

The sampling method will be a sequential non-probabilistic sampling. Newly diagnosed GBM subjects in the three ICO hospitals, who meet the above criteria, will be offered to participate in this study. Then, it will be based on a clinical sample of selected patients when they have been diagnosed of GBM and are about to begin the Stupp protocol.

All potential participants will receive an information sheet and an informed consent (ANNEX 5 & 6). They will be considered recruited for the study only after reading and signing these documents.

7.4. Sample size

To calculate the sample size, the application PASS 16.0.4 was used (ANNEX 2).

A two-sided logrank test with an overall sample size of 59 subjects (29 in the control group and 30 in the treatment group) achieves 80,6% power at a 0,050 significance level to detect a hazard ratio of 0,4368 when the proportion surviving in the control group is 0,6000. The study lasts for 36 time periods of which subject accrual (entry) occurs in the first 12 time periods. Accepting an alpha risk of 0.05 and a beta risk of a 0.2 in a bilateral contrast, we need 59 patients to demonstrate a difference equal or higher than 20%.

The accrual pattern across time periods is uniform (all periods equal). The proportion dropping out of the control group is 0.05. The proportion dropping out of the treatment group is 0.05. The proportion switching from the control group to another group with a survival proportion equal to that of the treatment group is 0.0000. The proportion switching from the treatment group to another group with a survival proportion equal to that of the control group is 0.0000.

In addition, for the calculation, we have used the one-year OS rate of the pilot study, the reference (19), which is 61%, and subsequently the proportion of one-year OS that we consider significant and plausible based on preclinical studies and the phase II study previously discussed, this being 80%. That is, with our clinical trial we believe that the results will be at least a 20% increase in OS at one year.

7.4.1. Estimated time of recruitment

As we calculated before, 59 patients are needed for this study.

Based on the existent bibliography, we assumed that the incidence of GBM in our population is 4.17 (95% CI 3.80-4.57) cases per 100.000 inhabitants/year. According to the existent bibliography and the actual incidence of this disease, we have seen that more than 80 patients are newly diagnosed GBM in the ICO hospitals. Considering that 10% of patient may not want to participate or will leave the study, we will need 12 months to recruit these 59 patients.

7.5. Study variables

Dependent variables:

- One-year overall survival rate after diagnosis

This is the principal dependent variable, because it is the main objective of this study. OS is defined as the time taken from the diagnosis of GBM to death due to any cause or last follow-up of the patient. For subjects who are still alive at the time of the study analysis or who were lost during follow-up, survival will be censored on the last recorded date that the subject was known to be alive.

- Time to progression (TTP)

It is defined as the time from the confirmed diagnosis to the progression data (recurrence in the case of completely resected disease) or death from any cause. It will be evaluated by brain MRI every 12 weeks (according to the hospital protocol) and the center neuroradiologist will inform it, and all aspects of the study will be blinded (patient treatment, clinical professionals and researcher evaluations). In all cases, progression *(Figure 8)* will be assessed using the criteria of the Response Assessment in Neuro-Oncology (RANO) Working Group (77)**.**

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Figure 8. Rano criterion for progression. CR: complete response. PR: partial response. SD: stable disease. PD: progressive disease. FLAIR: fluid-attenuated inversion recovery. NA: not applicable (77).

**Progression occurs when one of these criterions is present.*
† Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

Any subject without progressive disease observed within the study period will be censored on the date of the last tumor evaluation.

- Treatment tolerance

It will be assessed using a single question that will ask: “How well do you think you are dealing with treatment side effects that you might have been experiencing or are experiencing?”. Participants will answer in a four-level scale: very bad, bad, fairly well and very well. In the study it will be categorized in: poor (very bad and bad) or good treatment tolerance (fairly well and very well). Any adverse effects that generate poor tolerance will be evaluated by the medical oncologist, who will know if that effect is due to TMZ or cannabinoids.

Any adverse effect that is not due to TMZ and may be secondary to cannabinoids, will be graduated and measured with the Common Terminology Criteria for Adverse Events (CTCAE) (78). In this, there are available scales for the main adverse effects that may result from cannabinoids.

Every undesired effect must be assessed as serious or not serious. The *Agencia Española del Medicamento y Productos Sanitario* (AEMPS) understood as serious those situations that:

- Provoke death
- They threaten the patient's life
- Provoke your hospitalization, or prolong it
- They cause work or school disability
- Induce congenital defects
- Be clinically relevant

Independent variable:

- Treatment: placebo or cannabinoids (Sativex®: THC and CBD).

This is a qualitative dichotomous qualitative variable. It will be expressed by a percentage of patients who take placebo and patients who take cannabinoids. The two different interventions and follow-up procedures were explained above.

Covariates

Table 4. Covariates in the study.

Covariate	Type	Measure instrument	Categories or values
Age	Continuous quantitative variable	Clinical examination	≤50 years >50 years
Gender	Dichotomous nominal qualitative variable	Clinical examination	Male Female
ECOG/OMS performance status	Discrete quantitative variable	Clinical examination	0 1 2
Extent of resection**	Continuous quantitative variable	Center radiologist report	≥70% <70%
MGMT	Dichotomous qualitative variable	Center anatomopathologist report	Yes No
Corticosteroid treatment	Dichotomous qualitative variable	Clinical examination	Yes No
Antiepileptic treatment	Dichotomous qualitative variable	Clinical examination	Yes No

**After surgery, complete resection means the absence of tumor. It can be identified by MRI done between 24-72 hours after surgery. Patients can be classified in three groups depending on the type of resection: biopsy only, partial or complete resection.
If resection cannot be done, as we will explain later, a biopsy of the lesion will be performed. Histological confirmation is always necessary.

I have considered some of the covariates that may confuse my objective and I have not considered others that, although they influence survival, such as the BMI and comorbidities, are not related to my objective.

7.6. Data collection and procedures

All patients treated in the medical oncology department of the hospitals involved in this study will be asked to meet all the inclusion and none of the exclusion criteria to participate in this study. The patient must accept and give written consent after reading the information sheet.

Patients will come to us after the incidental discovery of a brain mass. This discovery may have been made by a doctor before a clinic suggestive of a GBM or in the study of other possible causes. We will consider the first visit when the patient comes to our consultation after the resection of the mass or biopsy, performed by a neuroradiologist specialized in the examination of the CNS, a brain MRI 24-72 hours after surgery and having the result of pathological anatomy made by an expert neuropathologist confirming the suspicion of malignancy. All the patients will be included in the study at the same moment: when they start the Stupp protocol. The patient follow-up will be the same for each one.

1st visit

After resection, or biopsy, in a period of less than 6 weeks, is when we must have the first visit with the patient. Within the first 4-6 weeks after surgery, another MRI should be done before starting medical treatment.

On the first visit we will analyse the entire medical history, asking for personal and family history, as well as all the details of the current GBM process. In addition, we will perform a Mini Mental State Examination (MMSE) (ANNEX 4) (79) to see the patient's neurocognitive state and assess their ECOG, to know their abilities and limitations of daily life activities. Later, we will explain what they treatment consists of, what is the guideline and method of

administration, the possible unwanted effects and we will establish a safety net with the patient explaining that in case he/she has any unusual symptoms go to the emergency department.

Next, we will explain to the patient what our study consists of, how cannabinoids have proved to be effective in preclinical studies and case series, as well as the beneficial effects of these for the treatment of GBM and possible unwanted effects. In case they agree to enter our study, we will request a complete analysis, or we will review the most recent ones (≤ 2 weeks before the participation to the study), including blood count, biochemistry, liver profile and renal function to verify that there are no exclusion criteria.

Once the treatment is decided and the patient agrees to enter the study, he or she will be cited a few days later with the results of the blood test. In the event that all inclusion and no one of exclusion criteria are met, I will randomize the patient, in one group or another, by random numbers. With randomization, all possible confounding factors will be equally distributed.

At this time, covariates will be collected in all patients, when the event occurs and at the end of the follow-up the dependent variables. All factors that have to be tested in the first visit and in the following control visits will be written in a document that will be given to the oncologists that take part in the study (ANNEX 3).

The patient will be given an information sheet about the strengths and weakness of cannabinoids with all the information of our study (ANNEX 5). After that, he will be asked to enter the study and sign the informed consent (ANNEX 6)**.** The decision of which treatment to follow will be made according to the patient preferences, as it is suggested in the *Sociedad Española de Oncología Médica* (SEOM) Guidelines, and according to the existent information in the 2 therapies. The neurooncologist should advise the patient and resolve the doubts that might have. The patient will only be considered recruited for the study after signing the written informed consent.

We will record the baseline data and co-variables (age, gender, ECOG/OMS performance status, extent of resection, corticosteroid treatment and antiepileptic treatment), and the patient will be considered part of the “cannabinoids treatment group” or group A (n=30), which will receive Stupp regimen plus cannabinoids, or part of the “non-cannabinoids group” or group B (n=29), which will be given the standard Stupp treatment plus placebo *(Figure 9)***.** Recall that patient assignment in one group or another will be done randomly by random numbers.

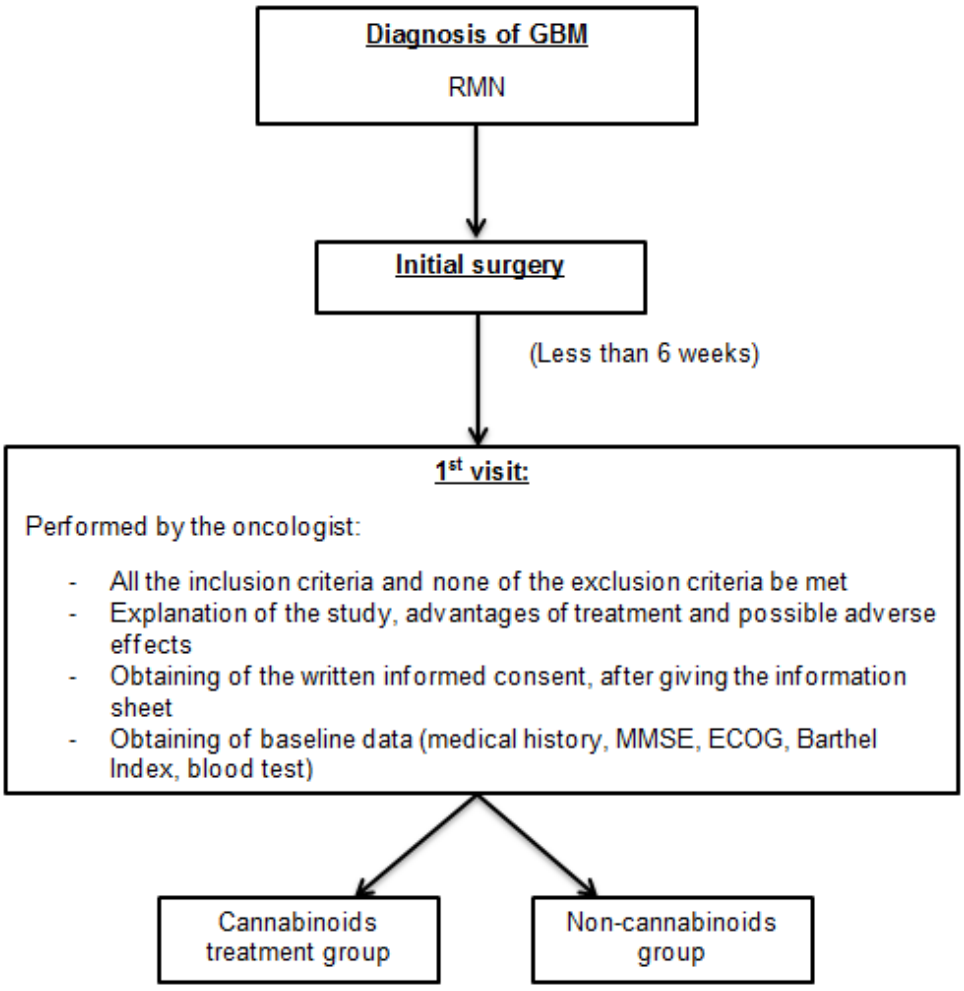


Figure 9. Flow chart of therapeutic decision

From this visit, the patient will begin concomitant treatment consisting of oral TMZ at a dose of 75 mg/m2 along with daily focal RT (except weekends) at a total dose of 60 Gy (2 Gy/day) for 6 weeks, a rest of 28 days and then adjuvant TMZ 150-200 mg/m2 x 5 days every 28 days until completing 6 cycles. In the event that the patient enters the group that receives the cannabinoids, Sativex®, these will be given every day, daily from the first visit to the progression, death or, failing that, the end of the study *(Figure 10)*. If, on the contrary, the patient enters in the "non-cannabinoids group", he will be given a placebo along with his/her treatment. The placebo used in this study will be a product with the same characteristics, shape, image and measures as the cannabinoid format, so it will be practically indistinguishable to the naked eye for patients and for any health or study staff.

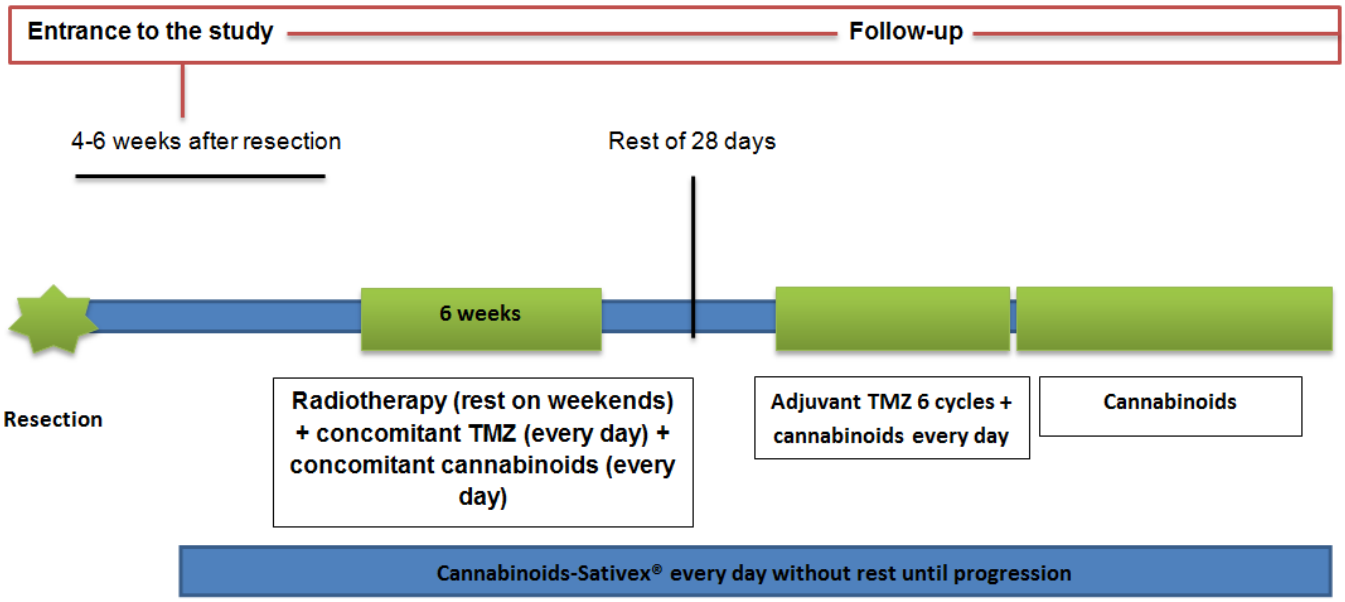


Figure 10. Study diagram.

TMZ is administered with ondansetron to prevent nausea and vomiting during adjuvant setting. After every meal, the patient waits 2 hours and takes the antiemetic. After 30 minutes, TMZ is administered. Once 1 hour has passed, the patient can eat again *(Figure 11)*. If ondansetron is contraindicated, another antiemetic will be administered (metoclopramide). This guideline will be followed both during the concomitance with RT and in the adjuvant.

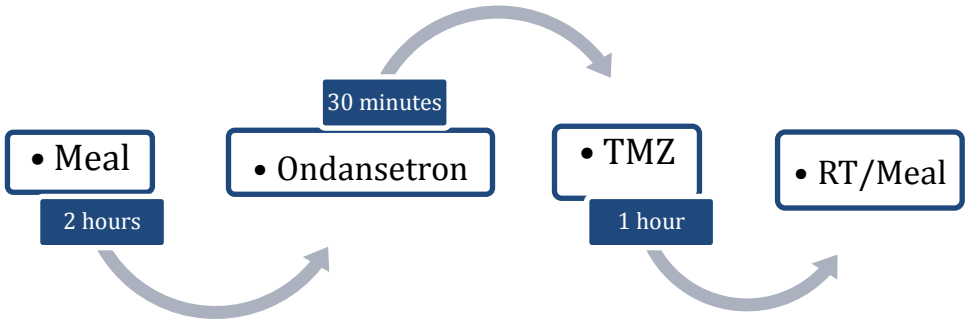


Figure 11.TMZ administration.

During the concomitant treatment phase RT will be given after taking de TMZ, at least one hour after taking TMZ. The reason why TMZ is first administered and then RT is that they produce a synergism: the spontaneous conversion of TMZ into the active metabolite and its ability to cross the blood – brain barrier is favoured by RT. (19).

Cannabinoids, or placebo, are administered by spraying. Sativex® is a solution for oral spraying: each spraying contains 100 microliters and it contains 2.7 mg of THC and 2.5 mg of CBD. The maximum number of sprays per day is 12, from which overdose may appear in the treated group. The subjects start with 6 sprays per day, going up through a dose escalation scheme based on efficacy and tolerability, increasing one spray per day each week during the visit (up to 12 sprays every 24 hours), thus being more control of adverse effects. The number of daily sprays is distributed in *table 5*. In the scenario where patients do not tolerate sprayings increase, we will stop at that step and he/she will remain with that dose until the end of the treatment. If the patient still does not tolerate it, the number of sprays will be decreased.

Being a phase II study, one of the purposes is to know the optimal dose as well as the safety (toxicity) and its therapeutic effect.

Table 5. Escalation of cannabinoids sprayings.

Week	Sprayings in de morning	Sprayings at night	Total sprayings per day
1	2	4	6
2	2	5	7
3	3	5	8
4	3	6	9
5	4	6	10
6	4	7	11
7	5	7	12

This guideline is based on evidence of the efficacy and effectiveness of cannabinoids in clinical trials of patients with multiple sclerosis (80,81), the only current indication of cannabinoids in the Spanish state, in preclinical and clinical trials in GBM and in the Sativex® data sheet (53,54)**.**

The subsequent management will be the same for all the patients in the study.

Subsequent visits

During the treatment, patients will have follow-up visits with the medical oncologist, in which the patient's condition and the possible unwanted effects during the entire cannabinoid treatment will be evaluated weekly as it is described below. The evolution of the tumor will be monitored with a brain MRI in the following way: after having performed an MRI prior to the start of medical treatment, the following one will be performed at the end of the concomitance (6 weeks later) and then one every 12 weeks until progression of the disease *(Figure 12)*. In addition, at each visit we request an MRI we will also perform an MMSE.

This image follow-up will be done in the same way for both the subjects of the treatment group and the control group.

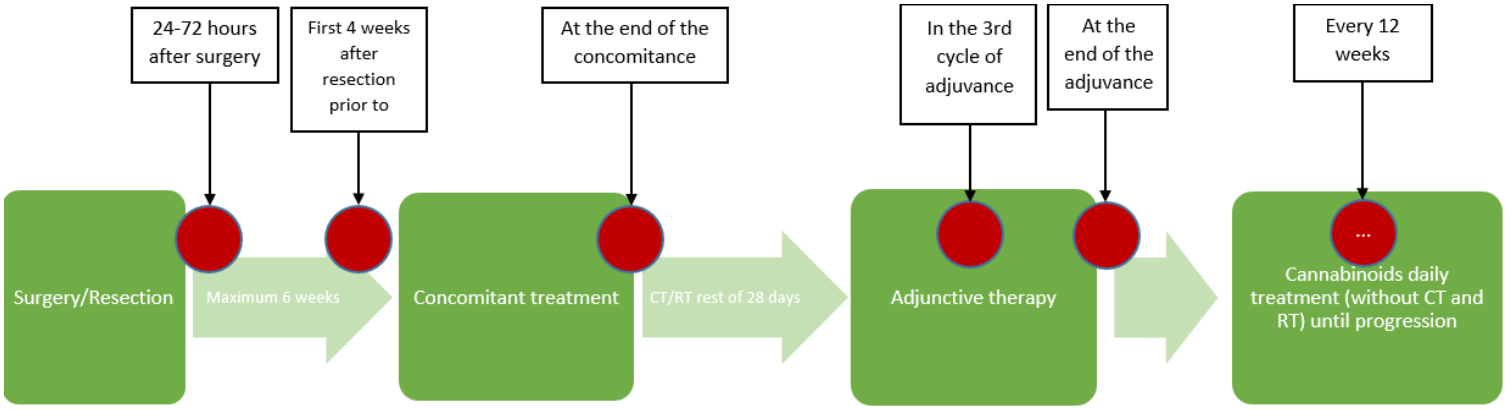


Figure 12. Brain MRI realization. Every red circle is a brain MRI and a MMSE.

After the first visit, being a phase II clinical trial with a new medication for patients, cannabinoids, and taking this concomitant with standard treatment, visits will be made once a week for the duration of RT treatment *(Figure 13)*. In each of the visits will be requested: a blood test that includes blood count, biochemistry, liver profile and renal function. At each visit, adverse events would be reported, the concomitant new medication, alterations of the blood test if they are related to the cannabinoids and the patient will be asked how he/she is doing, always assessing it with the ECOG (82) scale and the Barthel Index (83) (ANNEX 7 & 8) .

After the concomitance the patient rests 28 days of CT and has finished the treatment with RT, but will continue taking cannabinoids daily so during this period the subjects will be visited once a week, requesting the same as in the previous visits during the concomitance (blood test, alterations of this, adverse events, ECOG and Barthel Index).

When the adjuvant cycles of TMZ begin, a weekly visit will also be done, requesting the same analysis as the previous ones, reporting adverse events and blood test alterations in case they are related to the drug under study, in addition to analysing the ECOG and Barthel index of the subject under study.

Cannabinoid treatment should be followed until the patient has a progression of their disease (RANO criterion explained before), until the death and/or loss of the study patient. Thus, in this way, we can also take into account the data obtained in case we want to assess the free time to progression (PFS) in the future.

In the event that a patient reaches the end of the study follow-up without event or progression, the treatment would continue indefinitely until progression, regardless of whether the study has been completed and the statistical analysis begins.

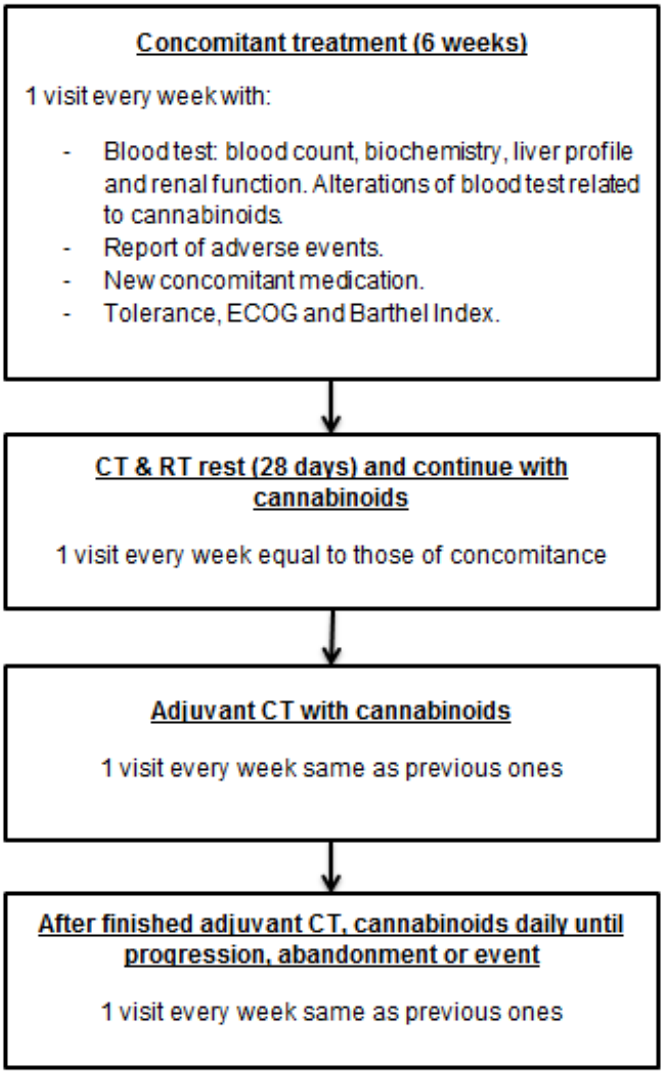


Figure 13. Flow char of patient's following.

Publicity measures will be carried out to prevent the loss of patients during follow-up. We will advertise our study in the media.

If any patient leaves the study, an attempt will be made to determine the reason for their abandonment and whether or not they leave the response variable, OS at one year, and/or time to progression.

Adverse effects

As we have explained above, adverse effects differ between serious and non-serious ones.

The known unwanted effects of Sativex® are listed in the table (ANNEX 9).

The *Sistema Español de Farmacovigilancia de Medicamentos de Uso Humano* will be notified by electronic form of any suspected adverse reaction not previously included in the Sativex® data sheet (84).

8. STATISTICAL ANALYSIS

8.1. Descriptive analysis

To summarize the dependent variable, treatment tolerance, I will perform it by proportions, stratifying by intervention and control groups.

The other two dependent variables, overall survival at one year and time to progression, will be summarized using medium and intercutaneous range, stratifying by intervention and control groups.

I will also use the Kaplan-Meier estimator to estimate the survival curves of these two dependent variables, one-year overall survival and time to progression, stratifying by intervention and control groups.

By randomizing, I have controlled much of the confounding but, if there is residual confusion, I will control this by the covariates previously described. To do this, I will stratify the previous analyses by the covariates. If a variable was quantitative I will categorize it in quartiles. Age will be categorized in less than ≤ 50 years old and older.

Covariates will be summarized in proportions (qualitative variables), means and standard deviations (continuous variables) and medians and interquartile range (discrete variables), stratifying by the groups of the intervention.

8.2. Bivariate inference

The association between the dependent variable tolerance to treatment and intervention, I will contrast it using the chi-square statistic. In the case that the cells have an expected number, Fisher's exact test will be used.

The difference between the survival curves for the variables dependent on one-year overall survival and time to progression will be checked by the two-sided Logrank test statistic.

Again, I will stratify the previous analyses by the covariates. In case the variable was quantitative, I will categorize it into quartiles. Age will be categorized in ≤ 50 and older than 50.

The difference in proportions (qualitative), in means (continuous quantitative) and in medians (discrete quantitative) of the covariates between the groups of the intervention will be tested using chi-square (or exact Fisher's test, when the expected counts were lower than 5), Student's t and Mann-Whitney's U test, respectively.

8.3. Mutivariate analysis

The association between the dependent variable tolerance to treatment and intervention will be adjusted in a logistic regression controlled by covariates.

The association between the other two dependent variables and the intervention will be assessed by a Cox regression controlled by the covariates.

9. ETHICAL AND LEGAL CONSIDERATIONS

Before carrying out the study, the research protocol will be presented to the Clinical Research Ethics Committee (CEIC) of Hospital Universitari Dr. Josep Trueta. Although this is a multicenter clinical trial, only the approval of one of the CEICs of the 3 centers is needed. If accepted, we will ask permission to perform it to the direction of the center and to the direction of the other ICO Hospitals involved in the study. ICO must also authorise the clinical trial.

Any input and contributions from the CEIC will be introduced in the study later.

Subsequently, when the addresses of the centers grant their agreement, authorization from the AEMPS will be requested *(Figure 14).*

In addition, it is necessary to purchase civil insurance, since the centers' civil liability policies are not enough.

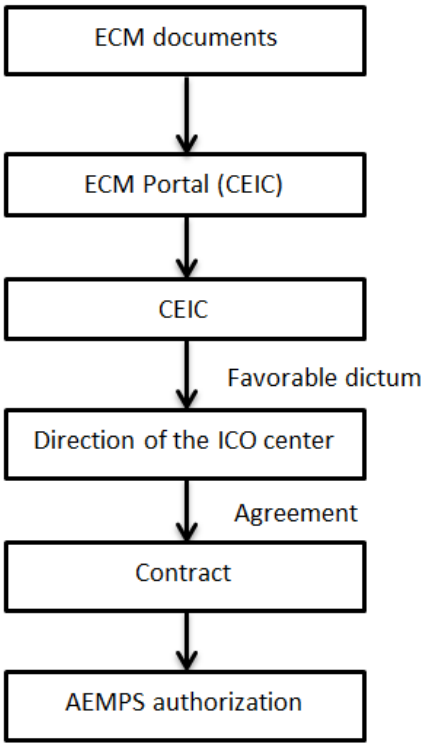


Figure 14. Flow chart of ethical procedure.

Since we will not depart from a previously constructed database, we will need the acquiescence of the patients to participate in the study. According to *Law 41/2002, de 14 de noviembre, Básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica*, every single patient – or their relatives, in case a patient would not be able to be informed for him/herself - will be properly informed of the aim, procedures, anticipated benefits, and potential hazards of the study. Prior to the beginning of the investigation, every subject participating in the clinical trial must be properly informed about the study to the fullest extent using language and terms they are able to understand in order to allow a fully knowledgeable decision.

At the time of the inclusion, they will be given an information sheet (ANNEX 5) with all the necessary information and an informed consent (ANNEX 6). They will only be a part of the study once they sign the written informed consent. A copy of the informed consent will be provided to the patient.

It will also be explained to the participants that they are free to refuse entry into the study and to withdraw from the study at any time without prejudice to future treatment. Patients at all-time have the right to leave the study with no impact on the quality of the health care that they will receive.

Ethically, the principles of biomedical and human research will be respected: principle of non-maleficence and respect for people, beneficence, patient autonomy and justice.

This study will be conducted according to the national and international ethics guidelines and laws:

- *WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects, June 1964. Last revision, October 2013*.
- *Real Decreto 1090/2015, de 4 de diciembre, de Investigación Biomédica*.
- *Real Decreto 1/2015 de 24 de julio, artículo 2*.
- *Ley orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales*. All the information and data collected from each patient during the course of the trial will be treated and used anonymously in order to guarantee and protect the public liberties and fundamental rights of persons. The medical record information, names and surnames will remain anonymous when publishing the results.

It will also be conducted with the fulfilment of the protocol, in accordance with ethical and methodological aspects of Good Clinical Practice guidelines in the European Union.

Subjects will be identified by their unique identification numeric code instead of their names. Personal patient data (personal identity and all personal medical information) will be maintained in privacy. In any presentation of the results of this study at conferences or publications, the patient identities will remain confidential. The data access will be only available for the research team, the Ethical and Clinical Investigation Committee, the pertinent health authorities and those responsible for data analysis.

The investigators of this project declare that there are no conflicts of interests, and that they do not receive any economic compensation to collaborate in the study.

10. STUDY LIMITATIONS

Collaborating hospitals

Some limitations of this study should be considered. It is done in three hospitals so it will be more expensive than if we did it in a single hospital. In addition to administrative, diagnostic, treatment and statistical expenses, we must hire a Contract Research Organization (CRO) in hospitals, which further increases the final expense. Information collection bias may occur, and it is difficult to standardize a protocol for all of the 3 hospitals and to control if the protocol is being well executed, so to prevent it, meetings every 6 months will be done to assure a correct sample collection, correct clinical information upload and correct protocol development in general. The CRO is needed as well as an initial visit with the principal investigator of each center, pharmacist and hospital data manager.

It is difficult for all the physicians responsible for each of the centers to analyse the situation of each patient in the same way or to decide if a worsening is a pseudoprogression, radionecrosis or tumor recurrence. Therefore, the international scales and indications in this study will be used to standardize all these variables, regardless of whether they are used in the hospital protocol.

Subsequently, another researcher will verify the agreement of the two previous researchers to avoid variability among the researchers.

Cannabinoids

Sativex®, despite the low concentration and low frequency of psychotropic effects that this could cause as well as the absence of dependence on the drug, can cause some adverse effects in patients. In addition, those patients with severe heart disease, pregnant women or who have a desire for pregnancy, a history of psychiatric disorders, as well as a history of substance abuse, should be excluded from the study and, therefore, cannot benefit from the drug. Likewise, the results of this study cannot be extrapolated to all those patients with any of these conditions.

Some patients may be reluctant to participate in the study by telling them that it is a drug, cannabinoids, but it must be explained that the concentrations of the drug are so small that they do not produce dependence or addiction and that the components act directly, although they do not exclusively, in cannabinoid tumor receptors. A correct explanation is necessary to

avoid the desire of the subjects not to participate only for fearing what is known as a drug. So, we do not really think that this is going to be a real limitation.

A very important limitation that must be taken into account is that Sativex® has the indication in other countries as antiemetic and that patients taking it may not require ondansetron. Therefore, it would no longer be a double blind because the researcher can guess it.

Sample collection

The sampling method will be a consecutive non-probabilistic, which has its own limitations; it has an implicit risk of selecting a non-representative sample. Consecutive method has been chosen because is one of the non-probabilistic methods that induces less bias. This type of sampling is useful for small sample sizes, and it is the best for uncommon pathologies, but it might not be representative of the whole population.

Confounding factors

In relation to confounding factors, it must be said that potential effect of them is possible but they are going to be considered in the statistical analysis and in the interpretation of results, so if we randomize and consider covariates, we hope we have no confounding.

Sample size

Despite being a prospective multicenter study with a time for the collection of samples of 12 months, we are aware that the sample may not seem very impressive, since 59 patients compared to other studies is little, but the calculation of the sample is accurate and sufficient to see an increase equal to or greater than 20% in OS at one year, which would be a great advance in the prognosis of an "orphan treatment" tumor.

With respect to other studies, retrospective and prospective, with statistically significant results and less sample than ours, we believe that the study has great potential.

Losses during follow-up

As this is a clinical trial and the GBM has a high mortality rate and low survival, the loss of patients during the follow-up is frequent. For this reason, the replacement of patients will be possible during the period of recruitment. The withdrawal will be recorded in order to avoid influencing in the study results and the internal validity of the study. However, it is important to note that the follow-up of this study does not differ from the habitual clinical practice, so no extra effort from the patients is required, with the exception of weekly apointments and blood samples between the end of RT and the begin of the adyuvant period.

In addition, we have calculated a sample size with a 10% drop-out rate, which we consider sufficient based on our knowledge and experiences of daily clinical practice, as well as literature, because the follow-up does not interfere in the patient's visits, as they are the same, so if the go to a medical revision, they will also be participating in the study follow-up. Also, no one, a part from the physician, will annoy them with questions or special requirements (unless they do not come) that could make them want to stand up for their right to abandon the study participation.

To avoid follow-up losses, telephone calls will be made to the patients or their relatives by the nurses/physician to know the reason for their absence and encourage them to continue in the study. Publicity measures will be carried out to prevent the loss of patients during follow-up.

Evaluation of the variables

There are possible information biases as, may be, not all the patients dependent (IDH status and MGMT promoter methylation) variables will be possible to analyse. That's because not all GBM are biopsied or surgically resected as it depends on the patient's general clinical state, among other (location of the tumor, concomitant diseases, high surgical risk…) that are not contemplated in this study. Although in the exclusion criteria those patients that have diseases that contraindicate surgery at diagnosis, in the evolution of GBM can appear other causes that make impossible the surgery.
It could also happen that there is a biopsy made but with insufficient material to analyse the molecular information and the patients' general state or the deep location of GBM contraindicates surgical intervention.
It is expected a low proportion of patients presenting this situation because in almost all cases at least a biopsy is made.

Duration and budget

Both depend on each other. The long duration of our study, 36 months, will hinder the search for funding and make its execution more expensive.

The financing will be achieved through research grants as the one from *Fondo de Investigaciones Sanitarias del Insituto de Salud Carlos III* or pharmaceutical financing as we will explain later.

Finally, we want to clarify that any correction from the CEIC will be introduced in the study later since this is a protocol and can be modified before the clinical trial begins.

11. FEASIBILITY

This study will be carried out in 3 third level ICO hospitals that already have all the means and resources available to accomplish the study. The study protocol and treatment of brain tumors, specifically GBM, is well established in ICO centers.

Additionally, most of the hospitals have already been contacted and agree with their collaboration in the study, which increases even more the feasibility in the recruiting of the needed sample.

The oncologists and investigators involved in the trial have enough experience in their specialty.

The number of expected patients participating in the study (59 patients in 3 different hospitals in 3 years) is affordable, from a logistic point of view.

Data collection is easy to do, since there are two key points:

- There is a well-structured database where all the clinical information and imaging and pathological studies of a patient are loaded and any doctor belonging to a hospital linked to it has access. This facilitates the collection of information relevant to the study.
- The patient will undergo all the diagnostic tests and periodic clinical reviews we need to perform this study in the public health system. All we need is to have access to patient information, blood tests and MRI.

We believe that the main obstacle in the execution of this protocol is the cost, and even so, we believe in its technical feasibility and in research grants to make it possible.

The work plan explains the steps that make the trial feasible.

12. WORKING PLAN AND CHRONOGRAM

The research team will develop the tasks of coordination, interpretation and presentation of the results. The principal investigators of our study will be the neurooncologists of each hospital and they will be the coordinator from each team, who will meet once every 6 months with the other coordinators.

The study will be multidisciplinary, considering as co-investigators the following team:

- A CRO staff
- A pharmacist staff from each hospital
- A neuroradiologist staff from each hospital
- A neuropathologist staff from each hospital
- A nursing staff from each hospital
- One statistic to analyze the results

The sequence of the activities will be developed in the following order:

Stage 0: Study design, coordination and training (December 2019 – February 2020).

1) Bibliographic research and protocol elaboration (objectives, hypothesis, variables and methodology).
2) First meeting of research team in order to meet the principal investigators of each hospital center included in our study. The organization of tasks and discussion of how to teach to fill the data information sheet will be also included.
3) Multidisciplinary team meetings and instructions to fill the data information sheet and sequence of data transference.
4) Training: the oncologists who will participate in the study will receive information about the study protocol (collecting and registering data, giving information to patients and diagnosing and treating GBM) in order to avoid differences when diagnosing and treating. That will ensure the homogeneity required to obtain representative conclusions.

Investigators and co-investigators will be the main responsible.

Stage 1: Ethical evaluation of the protocol (February 2020).

1) Presentation and evaluation of the protocol by the Clinical Research Ethics Committee of the *Hospital Universitari de Girona Doctor Josep Trueta,* Girona.
2) Contracting an insurance.

Investigators will be the main responsible.

Stage 2: Sample collection (March 2020– March 2021).

1) Patient recruitment: by a sequential non-probabilistic sequential sampling, patients will be enrolled in our study if they accomplish the inclusion and exclusion criteria and if they accept the informed consent. Patients will be randomly distributed in two groups by random numbers (control and treatment). Cannabinoids therapy will be administrated to one group according to the established plan.
2) Coordinators of each hospital will meet once every 6 months to evaluate if the protocol is being well fulfilled. If something does not work, they will take the necessary decisions.

Investigators and co-investigators will be the main responsible.

Stage 3. Data collection and follow-up visits. (March 2020-March 2023).

1) Follow-up visits: during the treatment, patients will be controlled once a week every week until progression, withdrawals or death.
2) Data collection: each neurooncologist will record the information collected in every visit in our database by using the data collection sheet (ANNEX 2). The database will be revised constantly to guarantee its functioning.

Investigators and co-investigators will be the main responsible.

Stage 4. Data analysis and interpretation (March 2023 – March 2024).

1) Construction and control of quality of the data base
2) Statistical analysis: performed by an experienced statistical. All the information collected will be analyzed by him or her according to the variables of our trial.
3) Interpretation of results: the principal investigators are the responsible. After this step, the discussion and conclusion will be elaborated.

Coordinators and the statistic are the main responsible.

Stage 5. Publication of results (March 2024 – April 2024).

1) Principal investigators will generate a paper to show the study results and conclusions. The results will not only be shown to the entire scientific community in the main congresses of neurooncology, they also will be attempted to publish.

Coordinators are the main responsible.

Table 6 and 7. Chronogram and study protocol.

YEAR	MONTH	ACTIVITIES
2019	December	**Stage 0**: Study design, coordination, meetings and training
2020	January	
	February	**Stage 1**: Ethical evaluation of the protocol by CEIC and contracting insurance
	March	
	April	
	May	
	June	
	July	
	August	**Stage 2**: Sample collection and coordination meetings
	September	
	October	
	November	
	December	
2021	January	
	February	
	March	
	April	
	May	
	June	
	July	
	August	
	September	**Stage 3**: Data collection and follow-up visits
	October	
	November	
	December	
2022	January	
	February	
	March	
	April	
	May	
	June	
	July	
	August	
	September	
	October	
	November	
	December	
2023	January	
	February	
	March	
	April	
	May	
	June	
	July	**Stage 4**: Construction and control of quality of the data base, statistical analysis and interpretation
	August	
	September	
	October	
	November	
	December	
2024	January	
	February	
	March	**Stage 5**: Publication of results
	April	

	SCREEN	RT & CT CONCOMITANT (6 WEEKS)						REST	ADJUVANT TMZ (6 CYCLES: CT 5 days every 28 days)						FOLLOW UP (every week until progression)
		1st	2nd	3rd	4th	5th	6th	28 days	1st	2nd	3rd	4th	5th	6th	
PROTOCOL ACTIVITY								EVERY WEEK							
Medical history and records	X														
Informed consent	X														
Tests:															
MRI	X						X				X			X	X (every 12 weeks)
Blood tests	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Questionnaires:															
ECOG	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MMSE	X						X				X			X	X (every 12 weeks)
Barthel	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Treatment tolerance	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

13. BUDGET

ITEM	QUANTITY	COST	SUBTOTAL
Personnel costs			
Qualified statistic	1 (150 hours)	70€/hour	10.500€
CRO	1	-	50.000€
Insurance policy			
Trial policy	1	20.000€/trial	20.000€
Material			
Sativex®	3 packages every 4 months for each patient (30 patients)	400€/pack	108.000€ (per year of treatment)
Placebo	3 packages every 4 months for each patient (29 patients)	5€/pack	1.305€ (per year of treatment)
Blood test	1 every week for each patient (59 patients)	6€/test	16.992€ (per year of treatment)
MMSE copies	Depend on the time and progression	0,10€/unit	60€ (per year of treatment)
Travels			
National congress (inscription fee, travel and accommodation)	1	1.800€	1.800€
International congress (inscription fee, travel and accommodation)	1	2.800€	2.800€
Publishing expenses and dissemination of results			
Meetings expenses	1 each 6 months (6 in total)	300€ each one	1.800€
Publication	1	English correction 700€ Open Access 1800€	2.500€
TOTAL			**215.757€**

Being a non-commercial clinical investigation, it will not be necessary to pay the AEMPS fees.

14. IMPACT ON THE NATIONAL HEALTH SYSTEM AND FUTURE PERSPECTIVES

GBM is an important tumor in terms of OS, mortality and life-threatening. For this reason, numerous important studies have been carried out worldwide to achieve an adequate treatment of the disease. In this sense, our study would help to establish a new exclusive oral therapeutic given with standard treatment that could improve the one-year OS rate.

Based on our hypothesis, the concomitant treatment with cannabinoids will improve not only the survival at one year, it will delay the disease progression and it is going to be well tolerated.

This is a multicenter study, so we will be able to generalize the results. We also have the opportunity to perform our study with the ICO, which has experience conducting many clinical trials. If the results are interesting, we will have the opportunity to continue widening our understanding of this disease with longer-term studies.

At the time of the present protocol, there has not been published any study that compares directly giving cannabinoids with standard GBM treatment or not giving them. It would be the first study testing it in Spain. With all this factors in mind, it would be a step forward in the treatment of this cancer.

15. PERSONAL REFLECTION

This study is a clinical trial whose costs may seem excessive. However, it is necessary to emphasize the importance that it would have if the results were positive, the impact on the survival of a cancer that is currently without hope and the “doors” that it would open in the investigation of this and other deceases. To deal with these expenses we have planned:

- A grant from the *Fondo de Investigaciones Sanitarias del Insituto de Salud Carlos III.*
- If not, request financing by the pharmaceutical industry (GW Pharmaceuticals).
- If not, request help from cannabis consumer associations in Netherlands (Sensi Seeds) to finance Sativex® or manufacture it in their laboratories.

It is important to emphasize that the total price of the study varies depending on the patient's follow-up: the more follow-up, the more cost. Not knowing what the prognosis of each patient is and what will happen to them, the total estimated cost is an annual cost.

16. CONFLICT OF INTERESTS

The authors declare no conflict of interest in any step of this trial.

17. REFERENCES

1. Etxeberria J, San Román E, Burgui R, Guevara M, Moreno-Iribas C, Urbina MJ, et al. Brain and central nervous system cancer incidence in Navarre (Spain), 1973-2008 and projections for 2014. J Cancer. 2015;6(2):177–83.
2. Contreras LE. Epidemiología de tumores cerebrales. Rev Médica Clínica Las Condes. 2017;28(3):332–8.
3. AECC. Evolución de los tumores del sistema nervioso central [Internet]. 2018 [cited 2019 Feb 5]. Available from: https://www.aecc.es/es/todo-sobre-cancer/tipos-cancer/sistema-nervioso-central/evolucion-tumores-sistema-nervioso-central
4. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer [Internet]. 2013 [cited 2019 Nov 5];49(6):1374–403. Available from: http://dx.doi.org/10.1016/j.ejca.2012.12.027
5. ECIS - European Cancer Information System. Incidence, mortality and prevalence of brain and CNS cancer in 2012 [Internet]. European Union; 2020 [cited 2019 Feb 5]. Available from: https://ecis.jrc.ec.europa.eu
6. Ostrom QT, Gittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C, et al. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2009-2013. Neuro Oncol. 2016;18:v1–75.
7. Pérez P. Tumores cerebrales [Internet]. SEOM. Madrid; 2019 [cited 2019 Feb 7]. Available from: https://seom.org/info-sobre-el-cancer/tumores-cerebrales
8. Nabors LB, Ammirati M, Bierman PJ, Brem H, Butowski N, Chamberlain MC, et al. Central Nervous System Cancers: Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw [Internet]. 2013 [cited 2019 Oct 13];11(9):1114–51. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4124889/
9. Frühwald MC, Rutkowski S. Tumors of the Central Nervous System in Children and Adolescents. Dtsch Arztebl. 2011;108(22):390–7.
10. Thakkar JP, Dolecek TA, Horbinski C, Ostrom QT, Lightner DD, Barnholtz-Sloan JS, et al. Epidemiologic and molecular prognostic review of glioblastoma. Cancer Epidemiol Biomarkers Prev. 2014;23(10):1985–96.
11. Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, et al. The epidemiology of glioma in adults: A state of the science review. Neuro Oncol. 2014;16(7):896–913.
12. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007;114(2):97–109.
13. Fuentes-Raspall R, Solans M, Roca-Barceló A, Vilardell L, Puigdemont M, del Barco S, et al. Descriptive epidemiology of primary malignant and non-malignant central nervous tumors in Spain: Results from the Girona Cancer Registry (1994–2013). Cancer Epidemiol. 2017;50:1–8.
14. Rory Goodwin C, Liang L, Abu-Bonsrah N, Hdeib A, Elder B, Kosztowski T, et al. Extraneural Glioblastoma Multiforme Vertebral Metastasis. World Neurosurg. 2016;89:578–82.
15. Pearson JRD, Regad T. Targeting cellular pathways in glioblastoma multiforme. Signal Transduct

Target Ther. 2017;2(June):1–11.

16. Herrera I, Gonzalez J, Garcia J, Juarez R, Ruano Y, Pinto J. Glioblastomas pirmarios y secundarios: ¿Es posible su diferenciación mediante parámetros radiológicos y biológicos? Seram. 2012;1–21.
17. An Z, Aksoy O, Zheng T, Fan QW, Weiss WA. Epidermal growth factor receptor and EGFRvIII in glioblastoma: Signaling pathways and targeted therapies. Oncogene [Internet]. 2018 [cited 2019 Jul 13];37(12):1561–75. Available from: http://dx.doi.org/10.1038/s41388-017-0045-7
18. Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. Clin Cancer Res. 2013;19(4):764–72.
19. Stupp R, Mason WP, Van Den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. N Engl J Med [Internet]. 2005 [cited 2019 Feb 5];352:987–96. Available from: www.nejm.org
20. Navone SE, Guarnaccia L, Locatelli M, Rampini P, Caroli M, La Verde N, et al. Significance and Prognostic Value of The Coagulation Profile in Patients with Glioblastoma: Implications for Personalized Therapy. World Neurosurg [Internet]. 2019 [cited 2019 May 14];121:e621–9. Available from: https://doi.org/10.1016/j.wneu.2018.09.177
21. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: A randomized phase III clinical trial. J Clin Oncol. 2013;31(32):4085–91.
22. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med. 2014;370(8):699–708.
23. Chinnaiyan P, Won M, Wen PY, Rojiani AM, Werner-Wasik M, Shih HA, et al. A randomized phase II study of everolimus in combination with chemoradiation in newly diagnosed glioblastoma: Results of NRG Oncology RTOG 0913. Neuro Oncol. 2018;20(5):666–73.
24. Stupp R, Taillibert S, Kanner A, Read W, Steinberg DM, Lhermitte B, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma a randomized clinical trial. JAMA - J Am Med Assoc. 2017;318(23):2306–16.
25. Kirson ED, Gurvich Z, Schneiderman R, Dekel E, Itzhaki A, Wasserman Y, et al. Disruption of Cancer Cell Replication by Alternating Electric Fields. Cancer Res. 2004;64(9):3288–95.
26. Shenouda G, Souhami L, Petrecca K, Owen S, Panet-Raymond V, Guiot MC, et al. A Phase 2 Trial of Neoadjuvant Temozolomide Followed by Hypofractionated Accelerated Radiation Therapy With Concurrent and Adjuvant Temozolomide for Patients With Glioblastoma. Int J Radiat Oncol Biol Phys [Internet]. 2017 [cited 2019 May 7];97(3):487–94. Available from: http://dx.doi.org/10.1016/j.ijrobp.2016.11.006
27. Chaudhry NS, Shah AH, Ferraro N, Snelling BM, Bregy A, Madhavan K, et al. Predictors of long-term survival in patients with glioblastoma multiforme: Advancements from the last quarter century. Cancer Invest. 2013;31(5):287–308.
28. Castañeda C, Casavilca S, Orrego E, García-Corrochano P, Deza P, Heinike H, et al. Glioblastoma : Análisis Molecular Y Glioblastoma : Molecular Analysis and Its Clinical. Rev Peru Med Exp Salud Publica. 2015;32(2):316–25.
29. Rosenfeld MR. Bridging Science and Clinical Practice: How to Use Molecular Markers When Caring for a Patient with Brain Cancer. In: Molecular markers in neuro-oncology. Alexandria, VA: ASCO Educational Book; 2013. p. 108–13.
30. Kaminska B, Czapski B, Guzik R, Król SK, Gielniewski B. Consequences of IDH1/2 mutations in gliomas and an assessment of inhibitors targeting mutated IDH proteins. Molecules. 2019;24(5):1–17.
31. Deng L, Xiong P, Luo Y, Bu X, Qian S, Zhong W, et al. Association between IDH1/2 mutations and

brain glioma grade. Oncol Lett. 2018;16(4):5405–9.

32. Lamborn KR, Chang SM, Prados MD. Prognostic factors for survival of patients with glioblastoma: Recursive partitioning analysis. Neuro Oncol. 2004;6(3):227–35.

33. Kristensen LS, Michaelsen SR, Dyrbye H, Aslan D, Grunnet K, Christensen JI, et al. Assessment of quantitative and allelic MGMT methylation patterns as a prognostic marker in glioblastoma. J Neuropathol Exp Neurol. 2016;75(3):246–55.

34. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. Am J Pathol. 2007;170(5):1445–53.

35. Johnson DR, Sawyer AM, Meyers CA, O'Neill BP, Wefel JS. Early measures of cognitive function predict survival in patients with newly diagnosed glioblastoma. Neuro Oncol. 2012;14(6):808–16.

36. Mauer M, Stupp R, Taphoorn MJB, Coens C, Osoba D, Marosi C, et al. The prognostic value of health-related quality-of-life data in predicting survival in glioblastoma cancer patients: Results from an international randomised phase III EORTC Brain Tumour and Radiation Oncology Groups, and NCIC Clinical Trials Group study. Br J Cancer. 2007;97(3):302–7.

37. SEIC. Guía Básica sobre los Cannabinoides. Madrid: Ministerio del Interior; 2002.

38. Petersen G, Moesgaard B, Schmid PC, Schmid HHO, Broholm H, Kosteljanetz M, et al. Endocannabinoid metabolism in human glioblastomas and meningiomas compared to human non-tumour brain tissue. J Neurochem. 2005;93(2):299–309.

39. Russo EB. Current therapeutic cannabis controversies and clinical trial design issues. Front Pharmacol. 2016;7(Sep.):1–19.

40. Russo EB. Cannabidiol Claims and Misconceptions. Trends Pharmacol Sci [Internet]. 2017 [cited 2019 Jun 28];38:198–201. Available from: http://dx.doi.org/10.1016/j.tips.2017.03.006

41. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev. 2002;54(2):161–202.

42. Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG. Cannabidiol displays unexpectedly high potency as an antagonist of CB 1 and CB 2 receptor agonists in vitro. Br J Pharmacol. 2007;150(5):613–23.

43. Pertwee RG. Pharmacological actions of cannabinoids. Handb Exp Pharmacol. 2005;168:1–51.

44. Abbassian H, Whalley BJ, Sheibani V, Shabani M. Cannabinoid type 1 receptor antagonism ameliorates harmaline-induced essential tremor in rat. Br J Pharmacol. 2016;173(22):3196–207.

45. Laun AS, Song ZH. GPR3 and GPR6, novel molecular targets for cannabidiol. Biochem Biophys Res Commun [Internet]. 2017 [cited 2019 Jun 28];490(1):17–21. Available from: http://dx.doi.org/10.1016/j.bbrc.2017.05.165

46. Espejo-Porras F, Fernández-Ruiz J, Pertwee RG, Mechoulam R, García C. Motor effects of the non-psychotropic phytocannabinoid cannabidiol that are mediated by 5-HT1A receptors. Neuropharmacology [Internet]. 2013 [cited 2019 Jul 2];75:155–63. Available from: http://dx.doi.org/10.1016/j.neuropharm.2013.07.024

47. Ellert-Miklaszewska A, Ciechomska I, Kaminska B. Cannabinoid Signaling in Glioma Cells. In: Advances in experimental Medicine and Biology. Basel: Springer Science; 2013. p. 209–20.

48. Schley M, Ständer S, Kerner J, Vajkoczy P, Schüpfer G, Dusch M, et al. Predominant CB2 receptor expression in endothelial cells of glioblastoma in humans. Brain Res Bull. 2009;79(5):333–7.

49. De Jesús ML, Hostalot C, Garibi JM, Sallés J, Meana JJ, Callado LF. Opposite changes in cannabinoid CB1 and CB2 receptor expression in human gliomas. Neurochem Int. 2010;56(6–7):829–33.

50. Ciaglia E, Torelli G, Pisanti S, Picardi P, D'Alessandro A, Laezza C, et al. Cannabinoid receptor CB1 regulates STAT3 activity and its expression dictates the responsiveness to SR141716 treatment in

human glioma patients' cells. Oncotarget. 2015;6(17):15464–81.

51. Wu X, Han L, Zhang X, Li L, Jiang C, Qiu Y, et al. Alteration of endocannabinoid system in human gliomas. J Neurochem. 2012;120(5):842–9.

52. Hui-Chen Lu KM. An introduction to the endogenous cannabinoid system Hui-Chen. Physiol Behav. 2017;176(3):139–48.

53. Guzmán M, Duarte MJ, Blázquez C, Ravina J, Rosa MC, Galve-Roperh I, et al. A pilot clinical study of Δ9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. Br J Cancer. 2006;95(2):197–203.

54. GW Pharmaceuticals Achieves Positive Results in Phase 2 Proof of Concept Study in Glioma [Internet]. Cambridge: GW Pharmaceuticals; 2017 [cited 2019 Apr 21]. Available from: www.gwpharm.com.

55. Rocha FCM, Dos Santos Júnior JG, Stefano SC, Da Silveira DX. Systematic review of the literature on clinical and experimental trials on the antitumor effects of cannabinoids in gliomas. J Neurooncol. 2014;116(1):11–24.

56. Sánchez C, Galve-Roperh I, Canova C, Brachet P, Guzmán M. Δ9-Tetrahydrocannabinol induces apoptosis in C6 glioma cells. FEBS Lett. 1998;436(1):6–10.

57. Dumitru CA, Sandalcioglu IE, Karsak M. Cannabinoids in glioblastoma therapy: New applications for old drugs. Front Mol Neurosci. 2018;11(May):1–7.

58. Carracedo A, Gironella M, Lorente M, Garcia S, Guzmán M, Velasco G, et al. Cannabinoids induce apoptosis of pancreatic tumor cells via endoplasmic reticulum stress-related genes. Cancer Res. 2006;66(13):6748–55.

59. Salazar M, Carracedo A, Salanueva ÍJ, Hernández-Tiedra S, Lorente M, Egia A, et al. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. J Clin Invest. 2009;119(5):1359–72.

60. Hernández-Tiedra S, Fabriàs G, Dávila D, Salanueva ÍJ, Casas J, Montes LR, et al. Dihydroceramide accumulation mediates cytotoxic autophagy of cancer cells via autolysosome destabilization. Autophagy. 2016;12(11):2213–29.

61. Massi P, Valenti M, Solinas M, Parolaro D. Molecular mechanisms involved in the antitumor activity of cannabinoids on gliomas: Role for oxidative stress. Cancers (Basel). 2010;2(2):1013–26.

62. Marcu JP, Christian RT, Lau D, Zielinski AJ, Horowitz MP, Lee J, et al. Cannabidiol enhances the inhibitory effects of Δ9- tetrahydrocannabinol on human glioblastoma cell proliferation and survival. Mol Cancer Ther. 2010;9(1):180–9.

63. Scott KA, Dennis JL, Dalgleish AG, Liu WM. Inhibiting heat shock proteins can potentiate the cytotoxic effect of cannabidiol in human glioma cells. Anticancer Res. 2015;35(11):5827–37.

64. Blázquez C, González-Feria L, Álvarez L, Haro A, Casanova ML, Guzmán M. Cannabinoids inhibit the vascular endothelial growth factor pathway in gliomas. Cancer Res. 2004;64(16):5617–23.

65. Blázquez C, Casanova ML, Planas A, Gómez T, Fernández-Aceñero MJ, Aragonés J, et al. Inhibition of tumor angiogenesis by cannabinoids. FASEB J. 2003;10:529–31.

66. Solinas M, Massi P, Cantelmo AR, Cattaneo MG, Cammarota R, Bartolini D, et al. Cannabidiol inhibits angiogenesis by multiple mechanisms. Br J Pharmacol. 2012;167(6):1218–31.

67. Galve-Roperh I, Sánchez C, Cortés M, Gómez del Pulgar T, Guzmán M, Izquierdo M. Anti-tumoral action of cannabinoids: Involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat Med [Internet]. 2000 [cited 2019 Mar 10];6(3):313–9. Available from: https://www-nature-com.e.bibl.liu.se/articles/nm0300_313.pdf

68. Galanti G, Fisher T, Kventsel I, Shoham J, Gallily R, Mechoulam R, et al. Δ9-Tetrahydrocannabinol

inhibits cell cycle progression by downregulation of E2F1 in human glioblastoma multiforme cells. Acta Oncol (Madr). 2008;47(6):1062–70.

69. Manini I, Caponnetto F, Bartolini A, Ius T, Mariuzzi L, Loreto C Di, et al. Role of microenvironment in glioma invasion: What we learned from in vitro models. Int J Mol Sci. 2018;19(1):1–32.

70. Solinas M, Massi P, Cinquina V, Valenti M, Bolognini D, Gariboldi M, et al. Cannabidiol, a Non-Psychoactive Cannabinoid Compound, Inhibits Proliferation and Invasion in U87-MG and T98G Glioma Cells through a Multitarget Effect. PLoS One. 2013;8(10):1–9.

71. Soroceanu L, Murase R, Limbad C, Singer E, Allison J, Adrados I, et al. Id-1 is a Key Transcriptional Regulator of Glioblastoma Aggressiveness and a Novel Therapeutic Target. Cancer Res. 2014;73(5):1559–69.

72. Carracedo A, Lorente M, Egia A, Blázquez C, García S, Giroux V, et al. The stress-regulated protein p8 mediates cannabinoid-induced apoptosis of tumor cells. Cancer Cell. 2006;9(4):301–12.

73. Di Marzo V, Melck D, Orlando P, Bisogno T, Zagoory O, Bifulco M, et al. Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells. Biochem J. 2001;358(1):249–55.

74. Munson AE, Harris LS, Friedman MA, Dewey WL, Carchman RA. Antineoplastic activity of cannabinoids. J Natl Cancer Inst. 1975;55(3):597–602.

75. Bifulco M, Laezza C, Pisanti S, Gazzerro P. Cannabinoids and cancer: Pros and cons of an antitumour strategy. Br J Pharmacol. 2006;148(2):123–35.

76. Gómez Del Pulgar T, De Ceballos M, Guzmán M, Velasco G. Cannabinoids protect astrocytes from ceramide-induced apoptosis through the phosphatidylinositol 3-kinase/protein kinase B pathway. J Biol Chem. 2002;277(39):36527–33.

77. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: Response assessment in neuro-oncology working group. J Clin Oncol. 2010;28(11):1963–72.

78. National Institute of Health, Institute NC. Common Terminology Criteria for Adverse Events (CTCAE) versión 5.0. 2017 [cited 2019 Nov 23]; Available from: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf

79. Mini Mental State Examination [Internet]. 1999 [cited 2020 Jan 22]. Available from: https://www.ics.gencat.cat/3clics/guies/30/img/minimentaldef.MMSE.pdf

80. Serpell MG, Notcutt W, Collin C. Sativex long-term use: An open-label trial in patients with spasticity due to multiple sclerosis. J Neurol. 2013;260(1):285–95.

81. Novotna A, Mares J, Ratcliffe S, Novakova I, Vachova M, Zapletalova O, et al. A randomized, double-blind, placebo-controlled, parallel-group, enriched-design study of nabiximols* (Sativex®), as add-on therapy, in subjects with refractory spasticity caused by multiple sclerosis. Eur J Neurol. 2011;18(9):1122–31.

82. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649–55.

83. Barrero Solís C, García Arrioja S, Ojeda Manzano A. Índice de Barthel (IB): Un instrumento esencial para la evaluación funcional y la rehabilitación. Plast y Restauración Neurológica. 2005;4:81–5.

84. AEMPS. Ficha técnica del Sativex [Internet]. Madrid; 2018 [cited 2019 Feb 19]. Available from: https://cima.aemps.es/cima/dochtml/ft/72544/FichaTecnica_72544.htm

18. ANNEXES

ANNEX 1: GRADING OF CNS TUMORS ACORDING TO 2016 CNS WHO

WHO grades of select CNS tumours	
Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	II
Anaplastic astrocytoma, IDH-mutant	III
Glioblastoma, IDH-wildtype	IV
Glioblastoma, IDH-mutant	IV
Diffuse midline glioma, H3 K27M–mutant	IV
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	II
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted	III
Other astrocytic tumours	
Pilocytic astrocytoma	I
Subependymal giant cell astrocytoma	I
Pleomorphic xanthoastrocytoma	II
Anaplastic pleomorphic xanthoastrocytoma	III
Ependymal tumours	
Subependymoma	I
Myxopapillary ependymoma	I
Ependymoma	II
Ependymoma, *RELA* fusion–positive	II or III
Anaplastic ependymoma	III
Other gliomas	
Angiocentric glioma	I
Chordoid glioma of third ventricle	II
Choroid plexus tumours	
Choroid plexus papilloma	I
Atypical choroid plexus papilloma	II
Choroid plexus carcinoma	III
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	I
Gangliocytoma	I
Ganglioglioma	I
Anaplastic ganglioglioma	III
Dysplastic gangliocytoma of cerebellum (Lhermitte–Duclos)	I
Desmoplastic infantile astrocytoma and ganglioglioma	I
Papillary glioneuronal tumour	I
Rosette-forming glioneuronal tumour	I
Central neurocytoma	II
Extraventricular neurocytoma	II
Cerebellar liponeurocytoma	II
Tumours of the pineal region	
Pineocytoma	I
Pineal parenchymal tumour of intermediate differentiation	II or III
Pineoblastoma	IV
Papillary tumour of the pineal region	II or III
Embryonal tumours	
Medulloblastoma (all subtypes)	IV
Embryonal tumour with multilayered rosettes, C19MC-altered	IV
Medulloepithelioma	IV
CNS embryonal tumour, NOS	IV
Atypical teratoid/rhabdoid tumour	IV
CNS embryonal tumour with rhabdoid features	IV
Tumours of the cranial and paraspinal nerves	
Schwannoma	I
Neurofibroma	I
Perineurioma	I
Malignant peripheral nerve sheath tumour (MPNST)	II, III or IV
Meningiomas	
Meningioma	I
Atypical meningioma	II
Anaplastic (malignant) meningioma	III
Mesenchymal, non-meningothelial tumours	
Solitary fibrous tumour / haemangiopericytoma	I, II or III
Haemangioblastoma	I
Tumours of the sellar region	
Craniopharyngioma	I
Granular cell tumour	I
Pituicytoma	I
Spindle cell oncocytoma	I

ANNEX 2: SAMPLE SIZE BY PASS 16.0.4

Logrank Tests

Summary Statements

A two-sided logrank test with an overall sample size of 59 subjects (29 in the control group and 30 in the treatment group) achieves 80,6% power at a 0,050 significance level to detect a hazard ratio of 0,4368 when the proportion surviving in the control group is 0,6000. The study lasts for 36 time periods of which subject accrual (entry) occurs in the first 12 time periods. The accrual pattern across time periods is uniform (all periods equal). The proportion dropping out of the control group is 0,0500. The proportion dropping out of the treatment group is 0,0500. The proportion switching from the control group to another group with a survival proportion equal to that of the treatment group is 0,0000. The proportion switching from the treatment group to another group with a survival proportion equal to that of the control group is 0,0000.

Procedure Input Settings

Autosaved Template File
C:\Users\77913159S\Documents\PASS 16\Procedure Templates\Autosave\Logrank Tests - Autosaved 2019_12_10-10_50_45.t396

Design Tab

Solve For:	Sample Size
Alternative Hypothesis:	Two-Sided
Power:	0,80
Alpha:	0,05
Group Allocation:	Equal (N1 = N2)
Input Type:	Proportion Surviving
S1 (Proportion Surviving - Control):	0,60
Treatment Group Parameter:	S2 (Proportion Surviving - Treatment)
S2 (Proportion Surviving - Treatment):	0,80
T0 (Survival Time):	1
Accrual Time (Integers Only):	12
Accrual Pattern:	Uniform or Equal
Total Time (Integers Only):	36
Controls Lost:	0,05
Treatments Lost:	0,05
Controls Switch to Treatments:	0,0
Treatments Switch to Controls:	0,0

Options Tab

Number of Intervals within a Time Period:	2000

Numeric Results for the Logrank Test in Terms of Sample Size

Alternative Hypothesis: Two-Sided

T0 = 1

Power	N1	N2	N	Haz Ratio (HR)	Ctrl Prop Surv (S1)	Trt Prop Surv (S2)	Acc-rual Pat'n	Acc-rual Time/ Total Time	Ctrl Loss	Trt Loss	Ctrl to Trt	Trt to Ctrl	Alpha	Beta
0,8063	29	30	59	0,4368	0,6000	0,8000	Equal	12 / 36	0,0500	0,0500	0,0000	0,0000	0,0500	0,1937

References

Lakatos, Edward. 1988. 'Sample Sizes Based on the Log-Rank Statistic in Complex Clinical Trials', Biometrics, Volume 44, March, pages 229-241.

Lakatos, Edward. 2002. 'Designing Complex Group Sequential Survival Trials', Statistics in Medicine, Volume 21, pages 1969-1989.

Report Definitions

Power is the probability of rejecting a false null hypothesis. Power should be close to one.

N1|N2|N are the sample sizes of the control group, treatment group, and both groups, respectively.

Hazard Ratio (HR) is the treatment group's hazard rate divided by the control group's hazard rate.

Proportion Surviving is the proportion surviving past time T0.

Accrual Time is the number of time periods (years or months) during which accrual takes place.

Total Time is the total number of time periods in the study. Follow-up time = (Total Time) - (Accrual Time).

Ctrl Loss is the proportion of the control group that is lost (drop out) during a single time period (year or month).

Trt Loss is the proportion of the treatment group that is lost (drop out) during a single time period (year or month).

Ctrl to Trt (drop in) is the proportion of the control group that switch to a group with a hazard rate equal to the treatment group.

Trt to Ctrl (noncompliance) is the proportion of the treatment group that switch to a group with a hazard rate equal to the control group.

Alpha is the probability of rejecting a true null hypothesis. It should be small.

Beta is the probability of accepting a false null hypothesis. It should be small.

Numeric Results for the Logrank Test in Terms of Events

Alternative Hypothesis: Two-Sided

T0 = 1

Power	Ctrl Evts (E1)	Trt Evts (E2)	Total Evts (E)	Haz Ratio (HR)	Ctrl Prop Surv (S1)	Trt Prop Surv (S2)	Acc-rual Pat'n	Acc-rual Time/ Total Time	Ctrl Loss	Trt Loss	Ctrl to Trt	Trt to Ctrl	Alpha	Beta
0,8063	26,8	24,0	50,8	0,4368	0,6000	0,8000	Equal	12 / 36	0,0500	0,0500	0,0000	0,0000	0,0500	0,1937

ANNEX 3: QUADERN DE RECOLLIDA DE DADES I COVARIABLES

Primera visita, dades bàsiques i visites successives

Participant nombre: _____

Hospital: _____

Nº Història clínica: _____ Data: ___/___/___

Edat: _____

Gènere: Home ☐ Dona ☐

ECOG: 0 ☐ 1 ☐ 2 ☐

Resecció: Sí ☐ No ☐

Si resecció, ompli els següents ítems: <70% ☐ ≥70% ☐

Percentatge de resecció aproximat: _____

Índex de massa corporal (kg/m^2): <25 ☐ 25-30 ☐ >30 ☐

Pes: _____

Alçada:_____

Tractament corticosteoride: Sí ☐ No ☐

Tractament antiepilèptic: Sí ☐ No ☐

Tolerància al tractament: Mal ☐ Bé ☐

Efectes indesitjats: _____

Progressió del GBM: Sí ☐ No ☐

Alguna altra comorbiditat i/o nou tractament concomitant:_____

_____

ANNEX 3: CUADERNO DE RECOGIDA DE DATOS Y COVARIABLES

Primera visita, datos básicos y visitas sucesivas

Participante número: _____

Hospital: _____

Nº Historia clínica: _____ Fecha: ___/___/___

Edad: _____

Género: Hombre ☐ Mujer ☐

ECOG: 0 ☐ 1 ☐ 2 ☐

Resección: Sí ☐ No ☐

Si resección, rellene los siguientes ítems: <70% ☐ ≥70% ☐

Porcentaje de resección aproximado: _____

Índice de masa corporal (kg/m^2): <25 ☐ 25-30 ☐ >3 ☐

Peso: _____

Altura:_____

Tratamiento corticosteoride: Sí ☐ No ☐

Tratamiento antiepiléptico: Sí ☐ No ☐

Tolerancia al tratamiento: Mal ☐ Bien ☐

Efectos indeseados: _____

Progresión del GBM: Sí ☐ No ☐

Alguna otra comorbilidad y/o nuevo tratamiento concomitante:_____

_____

ANNEX 4: MINI MENTAL STATE EXAMINATION

Basado en Folstein et al. (1975), Lobo et al. (1979)

Nombre: Varón [ ] Mujer [ ]
Fecha: F. nacimiento: Edad:
Estudios/Profesión: Núm. Historia:
Observaciones:

¿En qué año estamos? 0-1 ¿En qué estación? 0-1 ¿En qué día (fecha)? 0-1 ¿En qué mes? 0-1 ¿En qué día de la semana? 0-1	**ORIENTACIÓN TEMPORAL** (máx. 5)	
¿En qué hospital (o lugar) estamos? 0-1 ¿En qué piso (o planta, sala, servicio)? 0-1 ¿En qué pueblo (ciudad)? 0-1 ¿En qué provincia estamos? 0-1 ¿En qué país (o nación, autonomía)? 0-1	**ORIENTACIÓN ESPACIAL** (máx. 5)	
Nombre tres palabras peseta-caballo-manzana (o balón-bandera-árbol) a razón de 1 por segundo. Luego se pide al paciente que las repita. Esta primera repetición otorga la puntuación. Otorgue 1 punto por cada palabra correcta, pero continúe diciéndolas hasta que el sujeto repita las 3, hasta un máximo de 6 veces. Peseta 0-1 Caballo 0-1 Manzana 0-1 (Balón 0-1 Bandera 0-1 Árbol 0-1)	Núm. de repeticiones necesarias **FIJACIÓN RECUERDO** inmediato (máx. 3)	
Si tiene 30 euros y me va dando de tres en tres, ¿Cuántos le van quedando?. Detenga la prueba tras 5 sustracciones. Si el sujeto no puede realizar esta prueba, pídale que deletree la palabra MUNDO al revés. 30 0-1 27 0-1 24 0-1 21 0-1 18 0-1 (O 0-1 D 0-1 N 0-1 U 0-1 M 0-1)	**ATENCIÓN CÁLCULO** (máx. 5)	
Preguntar por las tres palabras mencionadas anteriormente. Peseta 0-1 Caballo 0-1 Manzana 0-1 (Balón 0-1 Bandera 0-1 Árbol 0-1)	**RECUERDO DIFERIDO** (máx. 3)	
DENOMINACIÓN. Mostrarle un lápiz o un bolígrafo y preguntar ¿qué es esto?. Hacer lo mismo con un reloj de pulsera, lápiz 0-1, reloj 0-1. **REPETICIÓN.** Pedirle que repita la frase: "ni sí, ni no, ni pero" (o "en un trigal había 5 perros") 0-1. **ÓRDENES.** Pedirle que siga la orden: "coja un papel con la mano derecha, dóblelo por la mitad, y póngalo en el suelo". Coge con la mano derecha 0-1 dobla por la mitad 0-1 pone en suelo 0-1. **LECTURA.** Escriba legiblemente en un papel "cierre los ojos". Pídale que lo lea y haga lo que dice la frase 0-1. **ESCRITURA.** Que escriba una frase (con sujeto y predicado) 0-1. **COPIA.** Dibuje 2 pentágonos intersectados y pida al sujeto que los copie tal cual. Para otorgar un punto deben estar presentes los 10 ángulos y la intersección 0-1.	**LENGUAJE** (máx. 9)	
Puntuaciones de referencia: 27 ó más: normal 24 ó menos: sospecha patológica 12-24: deterioro 9-12: demencia	**PUNTUACIÓN TOTAL** (máx. 30 puntos)	

a.e.g.(1999)

ANNEX 5: FULL D'INFORMACIÓ PER AL PACIENT

Títol de l'estudi:

Effect of cannabinoids on the survival of glioblastoma: a phase II multicenter randomized clinical trial

Estimat / a,

Aquest document està pensat per a donar tota la informació rellevant i ajudar a prendre la decisió terapèutica més adequada en cada cas.

El glioblastoma multiforme o GBM és el tumor cerebral primari maligne més freqüent en els adults. La seva incidència és baixa en comparació amb altres tumors, però no infraestimable. És un tumor que deriva d'unes cèl·lules pròpies del nostre sistema nerviós, els astròcits. Quan aquestes cèl·lules sofreixen canvis i alteren es tornen cancerígenes, moment en el qual produeixen els astrocitomas. Segons les seves característiques histològiques, es classifiquen en 4 graus, des del grau I fins al IV, de menor a major agressivitat respectivament.

El tractament actual es basa en la cirurgia que ja li han realitzat més quimioteràpia i radioteràpia. És important que entengui que aquest tractament no cura la malaltia, sinó que intenta alentir al màxim la progressió i augmentar el temps fins que el glioblastoma aparegui de nou.

- **Objectiu de l'estudi**

Actualment, la supervivència és menor a la d'altres tumors, per la qual cosa es necessiten nous fàrmacs i teràpies que augmentin el temps de supervivència amb el millor benestar possible.

L'objectiu d'aquest estudi és demostrar que afegint unes polvoritzacions bucals de cannabinoides cada dia, sense efectes psicotròpics com el de la "droga" coneguda, s'augmenti la supervivència global a l'any del glioblastoma. Secundàriament, s'avaluarà el temps a la progressió, que es defineix com el temps que transcorre des del diagnòstic fins al moment en el qual el tumor progressa seguint els criteris de *Response Assessment in Neuro-Oncology (RANO)*.

- **En què consistiria la seva participació?**

El seu tractament serà el mateix que l'estàndard realitzat actualment, el conegut com a règim Stupp que li ha explicat el seu neurooncòlog, amb l'única diferència que aleatòriament vostè serà inclòs en un grup A o B. En el grup A, el tractament consisteix en quimioteràpia, radioteràpia i cannabinoides polvoritzats; en el grup B, el tractament és el mateix però es canvien els cannabinoides per un altre flascó amb una substància inactiva. Ni vostè ni els

professionals sanitaris sabran quin medicament o quin grup li ha tocat. Així, ens assegurem un correcte funcionament de l'estudi.

És important que tingui clar que en cap moment se li donarà més prioritat a un grup que a un altre. Tampoc es realitza un seguiment diferent a uns pacients o a uns altres. Simplement es tracta d'afegir unes polvoritzacions al tractament habitual del glioblastoma.

Per a decidir si pot entrar o no en l'estudi, el seu oncòleg li realitzarà unes preguntes i sol·licitarà una analítica que inclogui l'hemograma, la bioquímica, la funció renal i el perfil hepàtic, a més li realitzarà un ECOG i l'escala de Barthel, que són uns qüestionaris per a valorar com es troba vostè i què pot fer en el seu dia a dia.

- **Cannabinoides**

Punts a favor:

- És un tractament indolor, còmode i fàcil d'administrar.
- Té molt pocs efectes adversos i la tolerància és molt bona.
- El tractament amb aquest fàrmac mai serà perjudicial ni disminuirà la seva supervivència, sempre serà beneficiós o neutral, però mai perjudicial.
- Nombrosos estudis preclínicos i algun clínic han demostrat un augment de la supervivència en afectats per glioblastoma.

Punts en contra:

- Rares vegades s'experimenten problemes psicotròpics, però podria donar-se el cas.
- El seu efecte sobre els fetus i els gàmetes (espermatozoides i òvuls) és perjudicial pel que durant aquest tractament no es recomana la reproducció.

És important que tingui clar el possibles efectes adversos que li pot provocar aquest tractament, sent els més freqüents: mareig, fatiga, astènia, anorèxia, augment de l'apetit, visió borrosa, vertigen, desorientació, restrenyiment, diarrea, boca seca o sensació de malestar general. No tenen per què produir-se cap d'aquests efectes; la gran part dels pacients toleren molt bé el tractament.

El tractament amb cannabinoides es continuarà fins a la progressió de la seva malaltia, i en aquest cas l'oncòleg mèdic li explicarà les següents alternatives, que són les mateixes que les dels pacients que no han participat en aquest estudi.

- **Després de decidir el tractament inicial, què hauria de fer?**

Una vegada comenci el tractament, vostè haurà d'assistir setmanalment a una visita de control amb el seu neurooncòlog. En aquesta se li demanarà una anàlisi de sang amb els principals paràmetres analítics necessaris per a l'estudi (hemograma, bioquímica, perfil hepàtic i funció renal) i el seu metge li preguntarà com se sent vostè, si nota algun símptoma nou o malestar, si ha iniciat algun tractament o està pendent d'iniciar-se i li farà unes preguntes bàsiques per a saber quin és el seu estat general.

A més, de tant en tant se li farà una ressonància magnètica cerebral, seguint el protocol de l'hospital i les guies clíniques recomanades. Aquestes dates les hi comunicarà el seu oncòleg a les visites, però es resumeix en: una ressonància magnètica a l'inici i al final de la concomitància de quimioterpia més radioteràpia i cannabinoides, una altra a la meitat de la adyuvancia de la quimioterpia i una més al final d'aquesta. Posteriorment, les ressonàncies magnètiques cerebrals es realitzaran segons ho consideri el seu oncòleg i els protocols vigents.

Totes les proves seran realitzades a l'hospital en el qual vostè és atès pel seu oncòleg, no haurà de desplaçar-se a un altre lloc ni tampoc se li serà permès realitzar les analítiques en el seu centre d'atenció primària habitual.

És important que acudeixi a les visites, ja que així és més fàcil ajudar amb el seu tractament i vetllar pel seu benestar. En cas que no pugui venir i prefereixi comunicar-se per telèfon, faci-ho saber al seu oncòleg perquè pugui contactar amb vostè. Recordi que pot venir acompanyat/a dels seus familiars, la qual cosa és molt recomanable.

L'estudi té una durada total de 36 mesos. Els 12 primers mesos és el període en el qual se li demana als pacients que participin en l'estudi si estan interessats. Els següents 24 mesos són en els quals es realitza el seguiment i tractament. Finalment, en acabar aquest període, es realitzarà una anàlisi de les dades obtingudes per un estadístic. Totes aquestes dades es posaran a la seva disposició i el seu oncòleg els hi explicarà detalladament.

- **Pòlissa d'assegurança**

El promotor de l'estudi disposa d'una pòlissa d'assegurança que s'ajusta a la legislació vigent i que li proporcionarà la compensació i indemnització corresponent en cas de detriment de la salut que pugui aparèixer a conseqüència de participar en l'assaig clínic, la qual cosa serà molt improbable.

- **Confidencialitat**

Les dades obtingudes dels participants són estrictament confidencials. El tractament, la comunicació i la cessió de les dades de caràcter personal de tots els subjectes participants s'ajustarà a la Llei orgànica 3/2018, de 5 de desembre, de Protecció de Dades Personals i garantia dels drets digitals. D'acord amb aquesta llei, vostè podrà exercir els drets d'accés, modificació, oposició i cancel·lació d'aquestes dades. Les dades recollides per a l'estudi estaran identificats mitjançant un codi i solo els investigadors podran relacionar aquestes dades els quals queden registrats en una base de dades. En cap cas apareixerà el seu nom en la publicació dels resultats. L'accés a la seva informació personal quedarà restringit als investigadors, autoritats sanitàries i al Comitè d'Ètica de Recerca Clínica, mantenint sempre la confidencialitat.

- **Compensació econòmica**

Els investigadors no obtindran benefici econòmic algun procedent d'aquest estudi. A més, vostè no rebrà remuneració pel fet de participar en aquest, tenint en compte que tampoc li suposarà cap despesa. A més, els medicaments de l'assaig clínic no hauran de ser pagats per vostè.

- **Canvi d'opinió**

La participació en l'estudi és voluntària i, per tant, els participants poden canviar d'opinió i abandonar l'estudi en qualsevol moment, sense necessitat de donar cap explicació i sense que això repercuteixi en la qualitat del tractament proporcionat.

De la mateixa forma, si els participants que decideixen abandonar el tractament i prefereixen continuar només amb el tractament estàndard, el podran fer en qualsevol moment.

- **Més informació**

En cas que tingui qualsevol dubte o vulgui més informació, pregunti a la seva neurooncòlog o contacti a través del telèfon que ell li proporcionarà. També pot consultar amb altres professionals sanitaris per a qualsevol dubte.

Al final de l'estudi, les dades i resultats obtinguts els hi explicarà el seu mèdic oncòleg, per a així poder entendre si ha estat beneficiós.

Amb tot això, li convidem a participar en el nostre estudi. Els resultats que s'obtinguin poden permetre millorar el tractament de futurs casos d'aquesta patologia, i resultar en un benefici directe per a nous pacients que es trobin en la seva situació actual.

ANNEX 5: HOJA DE INFORMACIÓN PARA EL PACIENTE

Título del estudio:

Effect of cannabinoids on the survival of glioblastoma: a phase II multicenter randomized clinical trial

Estimado / a,

Este documento está pensado para dar toda la información relevante y ayudar a tomar la decisión terapéutica más adecuada en cada caso.

El glioblastoma multiforme o GBM es el tumor cerebral primario maligno más frecuente en los adultos. Su incidencia es baja en comparación con otros tumores, pero no infraestimable. Es un tumor que deriva de unas células propias de nuestro sistema nervioso, los astrocitos. Cuando estas células sufren cambios y alteran se vuelven cancerígenas, momento en el que producen los astrocitomas. Según sus características histológicas, se clasifican en 4 grados, desde el grado I hasta el IV, de menor a mayor agresividad respectivamente.

El tratamiento actual se basa en la cirugía que ya le han realizado más quimioterapia y radioterapia. Es importante que entienda que este tratamiento no cura la enfermedad, sino que intenta ralentizar al máximo la progresión y aumentar el tiempo hasta que el glioblastoma aparezca de nuevo.

- **Objetivo del estudio**

Actualmente, la supervivencia es menor a la de otros tumores, por lo que se necesitan nuevos fármacos y terapias que aumenten el tiempo de supervivencia con el mejor bienestar posible.

El objetivo de este estudio es demostrar que añadiendo unas pulverizaciones bucales de cannabinoides cada día, sin efectos psicotrópicos como el de la "droga" conocida, se aumente la supervivencia global al año del glioblastoma. Secundariamente, se evaluará el tiempo a la progresión, que se define como el tiempo que transcurre desde el diagnóstico hasta el momento en el que el tumor progresa siguiendo los criterios de *Response Assessment in Neuro-Oncology (RANO)*.

- **¿En qué consistiría su participación?**

Su tratamiento será el mismo que el estándar realizado actualmente, el conocido como régimen Stupp que le ha explicado su neurooncólogo, con la única diferencia que aleatoriamente usted será incluido en un grupo A o B. En el grupo A, el tratamiento consiste en quimioterapia, radioterapia y cannabinoides pulverizados; en el grupo B, el tratamiento es el mismo pero se cambian los cannabinoides por otro frasco con una sustancia inactiva. Ni usted, ni los

profesionales sanitarios, sabrán qué medicamento o qué grupo le ha tocado. Así, nos aseguramos un correcto funcionamiento del estudio.

Es importante que tenga claro que en ningún momento se le dará más prioridad a un grupo que a otro. Tampoco se realiza un seguimiento diferente a unos pacientes o a otros. Simplemente se trata de añadir unas pulverizaciones al tratamiento habitual del glioblastoma.

Para decidir si puede entrar o no en el estudio, su oncólogo le realizará unas preguntas y solicitará una analítica que incluya el hemograma, la bioquímica, la función renal y el perfil hepático, y le realizará un ECOG y la escala de Barthel, que son unos cuestionarios para valorar cómo se encuentra usted y qué puede hacer en su día a día.

- **Cannabinoides**

Puntos a favor:

- Es un tratamiento indoloro, cómodo y fácil de administrar.
- Tiene muy pocos efectos adversos y la tolerancia es muy buena.
- El tratamiento con este fármaco nunca será perjudicial ni disminuirá su supervivencia, siempre será beneficioso o neutral, pero nunca perjudicial.
- Numerosos estudios preclínicos y alguno clínico han demostrado un aumento de la supervivencia en afectados por glioblastoma.

Puntos en contra:

- Rara vez se experimentan problemas psicotrópicos, pero podría darse el caso.
- Su efecto sobre los fetos y los gametos (espermatozoides y óvulos) es perjudicial por lo que durante este tratamiento no se recomienda la reproducción.

Es importante que tenga claro lo posibles efectos adversos que le puede provocar este tratamiento, siendo los más frecuentes: mareo, fatiga, astenia, anorexia, aumento del apetito, visión borrosa, vértigo, desorientación, estreñimiento, diarrea, boca seca o sensación de malestar general. No tienen por qué producirse ninguno de estos efectos; la gran parte de los pacientes toleran muy bien el tratamiento.

El tratamiento con cannabinoides se continuará hasta la progresión de su enfermedad, en cuyo caso el oncólogo médico le explicará las siguientes alternativas, que son las mismas que las de los pacientes que no han participado en este estudio.

- **Después de decidir el tratamiento inicial, qué debería hacer?**

Una vez comience el tratamiento, usted deberá asistir semanalmente a una visita de control con su neurooncólogo. En esta se le pedirá un análisis de sangre con los principales parámetros analíticos necesarios para el estudio (hemograma, bioquímica, perfil hepático y función renal) y su médico le preguntará cómo se siente usted, si nota algún síntoma nuevo o malestar, si ha iniciado algún tratamiento o está pendiente de iniciarse y le hará unas preguntas básicas para saber cuál es su estado general.

Además, de vez en cuando se le hará una resonancia magnética cerebral, siguiendo el protocolo del hospital y las guías clínicas recomendadas. Estas fechas se las comunicará su oncólogo a las visitas, pero se resume en: una resonancia magnética al inicio y al final de la concomitancia de quimioterapia más radioterapia y cannabinoides, otra a la mitad de la adyuvancia de la quimioterapia y una más al final de esta. Posteriormente, las resonancias magnéticas cerebrales se realizarán según lo considere su oncólogo y los protocolos vigentes.

Todas las pruebas serán realizadas en el hospital en el que usted es atendido por su oncólogo, no tendrá que desplazarse a otro lugar ni tampoco se le será permitido realizar las analíticas en su centro de atención primaria habitual.

Es importante que acuda a las visitas, ya que así es más fácil ayudar con su tratamiento y velar por su bienestar. En caso de que no pueda venir y prefiera comunicarse por teléfono, hágalo saber a su oncólogo para que pueda contactar con usted. Recuerde que puede venir acompañado/a de sus familiares, lo que es muy recomendable.

El estudio tiene una duración total de 36 meses. Los 12 primeros meses es el periodo en el que se le pide a los pacientes que participen en el estudio si están interesados. Los siguientes 24 meses son en los que se realiza el seguimiento y tratamiento. Finalmente, al acabar este periodo, se realizará un análisis de los datos obtenidos por un estadístico. Todos estos datos se pondrán a su disposición y su oncólogo se los explicará detalladamente.

- **Póliza de seguro**

El promotor del estudio dispone de una póliza de seguro que se ajusta a la legislación vigente y que le proporcionará la compensación e indemnización correspondiente en caso de detrimento de la salud que pueda aparecer como consecuencia de participar en el ensayo clínico, lo cual será muy improbable.

- **Confidencialidad**

Los datos obtenidos de los participantes son estrictamente confidenciales. El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los sujetos participantes se ajustará a la *Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales*. De acuerdo a ésta ley, usted podrá ejercer los derechos de acceso, modificación, oposición y cancelación de estos datos. Los datos recogidos para el estudio estarán identificados mediante un código y solo los investigadores podrán relacionar estos datos los cuales quedan registrados en una base de datos. En ningún caso aparecerá su nombre en la publicación de los resultados. El acceso a su información personal quedará restringido a los investigadores, autoridades sanitarias y al Comité de Ética de Investigación Clínica, manteniendo siempre la confidencialidad.

- **Compensación económica**

Los investigadores no obtendrán beneficio económico alguno procedente de este estudio. Además, usted no recibirá remuneración por el hecho de participar en el mismo, teniendo en cuenta que tampoco le supondrá ningún gasto. Además, los medicamentos del ensayo clínico no tendrán que ser pagados por usted.

- **Cambio de opinión**

La participación en el estudio es voluntaria y, por tanto, los participantes pueden cambiar de opinión y abandonar el estudio en cualquier momento, sin necesidad de dar ninguna explicación y sin que ello repercuta en la calidad del tratamiento proporcionado.

De la misma forma, si los participantes que deciden abandonar el tratamiento y prefieren continuar sólo con el tratamiento estándar, lo podrán hacer en cualquier momento.

- **Más información**

En caso de que tenga cualquier duda o quiera más información, pregunte a su neurooncólogo o contacte a través del teléfono que él le proporcionará. También puede consultar con otros profesionales sanitarios para cualquier duda. Al final del estudio, los datos y resultados obtenidos se los explicará su médico oncólogo, para así poder entender si ha sido beneficioso.

Con todo ello, le invitamos a participar en nuestro estudio. Los resultados que se obtengan pueden permitir mejorar el tratamiento de futuros casos de esta patología, y resultar en un beneficio directo para nuevos pacientes que se encuentren en su situación actual.

ANNEX 6: CONSENTIIMENT INFORMAT

Títol de l'estudi:

Effect of cannabinoids on the survival of glioblastoma: a phase II multicenter randomized clinical trial

Jo (nom i cognoms),_____, amb DNI _____, data de naixement _____ i pacient del/de la Dr./Dra. _____confirmo que:

- He rebut i llegit el full d'informació per al pacient que se m'ha entregat
- He pogut fer preguntes sobre l'estudi i els meus dubtes han estat resolts
- He rebut suficient informació sobre l'estudi
- He sigut informat/da de les implicacions i objectius del estudi i entenc quin serà el meu paper com a participant de l'estudi
- Entenc que les meves dades seran tractades de forma estrictament confidencial respectant la meva confidencialitat
- Comprenc que la meva participació és voluntària i que puc retirar-me de l'estudi quan vulgui, podent revocar el consentiment sense que això repercuteixi en la meva assistència sanitària futura

En conseqüència, dono la meva conformitat a participar en l'estudi "*Effect of cannabinoids on the survival of glioblastoma: a phase II multicenter randomized clinical trial"* i estic d'acord en què la informació obtinguda en aquest assaig clínic pugui ser utilitzada en investigacions futures sobre el tractament del glioblastoma multiforme.

	Número de telèfon	Correu electrònic
Medis de contacte:	_____	_____
		_____

Firma del participant:

_____

Data: ____ / ____ / ____

Firma de l'investigador:

_____

Data: ____ / ____/ ____

REVOCACIÓ DEL CONSENTIMENT INFORMAT

Jo (nom i cognoms), _____, revoco el consentiment de participació a l'estudi a sobre indicat.

Firma del pacient: Data: ___ / ___ / ___

_____

ANNEX 6: CONSENTIMIENTO INFORMADO

Título del estudio:

Effect of cannabinoids on the survival of glioblastoma: a phase II multicenter randomized clinical trial

Yo (nombre y apellidos),_____, con DNI _____, fecha de nacimiento _____ y paciente del/de la Dr./Dra. _____confirmo que:

- He recibido y leído la hoja de información para el paciente que se me ha entregado
- He podido hacer preguntas sobre el estudio y mis dudas han sido resueltos
- He recibido suficiente información sobre el estudio
- He sido informado/a de las implicaciones y objetivos del estudio y entiendo cuál será mi papel como participante del estudio
- Entiendo que mis datos serán tratados de forma estrictamente confidencial respetando mi confidencialidad
- Comprendo que mi participación es voluntaria y que puedo retirarme del estudio cuando quiera, pudiendo revocar el consentimiento sin que ello repercuta en mi asistencia sanitaria futura

En consecuencia, doy mi conformidad a participar en el estudio "*Effect of cannabinoids on the survival of glioblastoma: a phase II multicenter randomized clinical trial"* y estoy de acuerdo en que la información obtenida en este ensayo clínico pueda ser utilizada en investigaciones futuras sobre el tratamiento del glioblastoma multiforme.

	Número de teléfono	Correo electrónico
Medios de contacto:	_____	_____
		_____

Firma del participante: Firma del investigador:

_____ _____

Fecha: ____ / ____ / ____ Fecha: ____ / ____/ ____

REVOCACIÓN DEL CONSENTIMIENTO INFORMADO

Yo (nombre y apellidos), _____, revoco el consentimiento de participación en el estudio indicado arriba.

Firma del paciente: Fecha: ___ / ___ / ____

_____

ANNEX 7: ECOG SCALE OF PERFORMANCE STATUS

• ECOG 0: El paciente se encuentra totalmente asintomático y es capaz de realizar un trabajo y actividades normales de la vida diaria.
• ECOG 1: El paciente presenta síntomas que le impiden realizar trabajos arduos, aunque se desempeña normalmente en sus actividades cotidianas y en trabajos ligeros. El paciente sólo permanece en la cama durante las horas de sueño nocturno.
• ECOG 2: El paciente no es capaz de desempeñar ningún trabajo, se encuentra con síntomas que le obligan a permanecer en la cama durante varias horas al día, además de las de la noche, pero que no superan el 50% del día. El individuo satisface la mayoría de sus necesidades personales solo.
• ECOG 3: El paciente necesita estar encamado más de la mitad del día por la presencia de síntomas. Necesita ayuda para la mayoría de las actividades de la vida diaria como por ejemplo el vestirse.
• ECOG 4: El paciente permanece encamado el 100% del día y necesita ayuda para todas las actividades de la vida diaria, como por ejemplo la higiene corporal, la movilización en la cama e incluso la alimentación.
• ECOG 5: Paciente fallecido.

ANNEX 8: BARTHEL INDEX

Parámetro y situación del paciente	**Puntuación**
Comer	
- Totalmente independiente	10
- Necesita ayuda para cortar carne, el pan, etc.	5
- Dependiente	0
Lavarse	
- Independiente: entra y sale solo del baño	5
- Dependiente	0
Vestirse	
- Independiente: capaz de ponerse y de quitarse la ropa, abotonarse, atarse los zapatos	10
- Necesita ayuda	5
- Dependiente	0
Arreglarse	
- Independiente para lavarse la cara, las manos, peinarse, afeitarse, maquillarse, etc.	5
- Dependiente	0
Deposiciones (valórese la semana previa)	
- Continencia normal	10
- Ocasionalmente algún episodio de incontinencia, o necesita ayuda para administrarse supositorios o lavativas	5
- Incontinencia	0

Micción (valórese la semana previa)	
- Continencia normal, o es capaz de cuidarse de la sonda si tiene una puesta	10
- Un episodio diario como máximo de incontinencia, o necesita ayuda para cuidar de la sonda	5
- Incontinencia	0
Usar el retrete	
- Independiente para ir al cuarto de aseo, quitarse y ponerse la ropa…	10
- Necesita ayuda para ir al retrete, pero se limpia solo	5
- Dependiente	0
Trasladarse	
- Independiente para ir del sillón a la cama	15
- Mínima ayuda física o supervisión para hacerlo	10
- Necesita gran ayuda, pero es capaz de mantenerse sentado solo	5
- Dependiente	0
Deambular	
- Independiente, camina solo 50 metros	15
- Necesita ayuda física o supervisión para caminar 50 metros	10
- Independiente en silla de ruedas sin ayuda	5
- Dependiente	0
Escalones	
- Independiente para bajar y subir escaleras	10
- Necesita ayuda física o supervisión para hacerlo	5
- Dependiente	0
TOTAL……	

Máxima puntuación: 100 puntos (90 si va en silla de ruedas)	
Resultado	**Grado de dependencia**
< 20	Total
20-35	Grave
40-55	Moderado
≥ 60	Leve
100	Independiente

Adapted from (83).

ANNEX 9: SATIVEX® ADVERSE EFFECTS

Clasificación de órganos del sistema MedDRA	**Muy frecuentes ≥ 1/10**	**Frecuentes de ≥ 1/100 a < 1/10**	**Poco frecuentes de ≥ 1/1.000 a < 1/100**
Infecciones e infestaciones			faringitis
Trastornos del metabolismo y de la nutrición		anorexia (incluyendo apetito disminuido), aumento del apetito	
Trastornos psiquiátricos		depresión, desorientación, disociación, estado de ánimo eufórico	alucinación (no especificadas, auditivas, visuales), ilusiones, paranoia, ideación suicida, percepción delirante*
Trastornos del sistema nervioso	mareos	amnesia, alteración del equilibrio, alteración de la atención, disartria, disgeusia, letargia, alteración de la memoria, somnolencia	síncope
Trastornos oculares		visión borrosa	
Trastornos del oído y del laberinto		Vértigo	
Trastornos cardíacos			palpitaciones, taquicardia
Trastornos vasculares			hipertensión
Trastornos respiratorios, torácicos y mediastínicos			irritación de garganta
Trastornos gastrointestinales		estreñimiento, diarrea, boca seca, glosodinia, ulceración de la boca, náuseas, molestias en la boca, dolor bucal, vómitos	dolor abdominal (superior), cambio de color de la mucosa oral*, alteración oral, exfoliación de la mucosa oral*, estomatitis, cambio de color de los dientes
Trastornos generales y alteraciones en el lugar de administración	fatiga	dolor en la zona de aplicación, astenia, sensación anormal, sensación de embriaguez, malestar general	irritación en la zona de aplicación
Lesiones traumáticas, intoxicaciones y complicaciones de procedimientos terapéuticos		caída	

* notificada en estudios abiertos a largo plazo

Adapted from (84).