RESEARCH PROTOCOL

Safety and feasibility of the use of cryoablation in patients with brain tumors

Erasmus MC, Department of Neurosurgery

PROTOCOL TITLE

Safety and feasibility of the use of cryoablation in patients with brain neoplasm

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: There are only limited surgical treatment options like microneurosurgery or biopsy for brain tumors. However, very recently several cases have been presented in the literature using a cryoablation technique. Cryoablation offers precise and safe lesion targeting with minimal blood loss, is minimally invasive and the ice-cone formation within the tumor can be monitored by several imaging techniques. Cryoablation could also potentially be used for non-resectable tumors. Furthermore, cryoablation could have a potential immunological effect in some malignant brain tumors. Cryoablation could thus be of high value in the minimal invasive treatment of several brain tumors.

Objective: This study will be performed to investigate the safety and feasibility of cryoablation during operation in adults in several brain tumors like gliomas, meningiomas, and brain metastasis. The primary objective is to assess safety in terms of complications rate & morbidity and feasibility, such as operation time, blood loss during intervention and practicability. The secondary objective is to analyse the confirmation of reaching macroscopic tumor edges using intraoperative ultrasound, progression free survival, and overall survival during the entire study period.

Study design: This study is set up as an open label, prospective pilot study (Phase I study). The study will include 30 patients with gliomas, meningiomas or brain metastasis at the ErasmusMC brain tumor center. In all patients, the tumor will be operated conform standard procedure in the operation room 2 (Research OR). Upon exposure of the tumor, a biopsy will be taken. Subsequently, one or a number of cryoprobes required for tumor ablation will be inserted into the tumor. After proper positioning, the cryoablation will start. The ice formation will be monitored continuously, using cone-beam CT and/or intraoperative ultrasound. Prior to cryoprobe placement, there will be one scan. During cryoprobe placement, a variable number of scans may be necessary. After cryoprobe placement, there will be one scan to confirm the position. Additionally, there will be one scan during each freezing cycle (1 or 2 cycles), and one scan after removing the cryoprobes. The duration of each freezing cycle will be a maximum of 10 minutes, followed by active thawing. After resection of the necrotic tumor, the patients will receive the standard treatment and follow up.

Study population: Adults with presumed gliomas (1. astrocytoma, IDH mutant 2. oligodendroglioma, IDH-mutant and 1p/19q-codeleted 3. glioblastoma, IDH-wildtype), meningiomas (WHO gr. 1 and gr 2) , or brain metastasis based on preliminary diagnosis.

Intervention (if applicable): See study design.

Main study parameters/endpoints: The primary outcome is to assess the safety and feasibility of cryoablation in patients with brain neoplasm. Safety will be expressed in terms of severity and frequency of the following complications: postoperative intracranial bleeding, wound infection, epilepsy, brain edema, neurological deficit, and aphasia. The feasibility will be expressed in terms of operation time (in minutes), blood loss during the intervention (in milliliters), and practicability. The primary outcome parameters will be compared to standard surgery.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: All patients included in this study will receive the same (neo-)adjuvant therapy (if necessary) as patients who did not participate in this study. Depending on the size of the tumor, cryoablation takes approximately 30 minutes to complete. However, we expect that the total operating time will be considerably shorter as the cryoablation effect on the tumor should shorten the tumor resection time. Cryoablation is already used for the treatment of prostate carcinoma, kidney carcinoma and several lung cancers often with better results than other surgical treatments. Although only few clinical cases have been published, cryoablation seems not to increase complications or mortality rates in brain surgery.
Patient with brain tumors could benefit significantly from this therapy in terms of operation time and blood loss. Cryoablation could also potentially be used for non-resectable tumors or very vascularized tumors. Furthermore, the supposed immunological effect could be very interesting in combination with adjuvant therapies in the future.

1. INTRODUCTION AND RATIONALE

The most common types of brain neoplasm are gliomas, meningioma's and brain metastasis. Less common types are vascular tumors such as hemangioblastoma's and cavernous hemangiomas. Frequent clinical presentation of brain tumors in general are seizure, cognitive dysfunction, headache and fatigue (1). The diagnosis and treatment response is largely evaluated using MRI. Neuroimaging plays an important role in the decision-making process for therapy (2). Depending on many factors, such as the type, size, location of the tumor, symptoms and the general health of the patient the main treatment of brain neoplasm is usually surgical resection or biopsy followed by radiotherapy and or chemotherapy if necessary (3-5). Surgical resection of brain tumors are usually long operations with relative high blood loss and high morbidity as compared to non-neurosurgical tumors. The development of new minimally invasive safe techniques are therefore warranted.

Recently, several cases have been published by others using a cryoablation technique for the treatment of tumors. Cryoablation uses freezing temperatures to destroy tumor and is already an accepted therapy for a variety of tumors like prostate carcinoma, kidney carcinoma and several lung cancers often with better results than other surgical treatments (6-13). Cryoablation offers precise and safe lesion targeting, is minimally invasive and the ice-cone formation within the tumor can be monitored by several imaging techniques. Furthermore, cryoablation could have a potential immunological effect in some malignant brain tumors. The sharp delineation between tumor and healthy tissue is a big advantage as compared to other ablation technique as RF and laser, which makes the treatment of brain tumors in eloquent areas with this technique very attractive for neurosurgeons. In other techniques as RF and laser ablation, the neurosurgeon cannot properly visualize the ablation during the process.

Due to the development of microsurgery and neuroanesthesiology, the clinical applications of early crude cryoablation of brain neoplasm, studied mainly in the 60s and 70s were phased out (14-19). Only three recent studies reported the use of cryoablation for gliomas and one study, for meningioma's, brain metastasis, and vascular tumors with good results (16, 17, 19, 20, 22). Martynov et al. (22) treated 88 patients with supratentorial gliomas considered not suitable for aggressive surgical resection, and underwent cryo destruction. The complication rate of the authors was 11,3% and the postoperative mortality was 1,1%. The complications and survival rate of this study corresponded with surgical resection of gliomas in eloquent areas in their clinic (14, 15). However, the study of Martynov et al. was performed by military surgeons with a homemade cryosystem and therefore the result could not be extrapolated. Gangi et al. (17) treated 4 patients with recurrent glioblastoma using cryoablation. In this study no complications were observed. Cebulla et al. (16) also treated patients with glioblastoma using cryoablation. One of the six patients that was treated developed transient paresis of elevator eyelid muscle. Both Martynov et al. and Gangi et al. froze the tumor twice, a so-called double freeze cycle. In contrast to Gangi et al. and Martynov et al. Cebulla et al. had only one freeze cycle. Surgical complications following malignant brain tumor surgery

has an occurance of 36.2 events per 1000 cases (24). In conclusion, cryoablation shows a comparable complication rate as standard brain tumor surgery.

Cryoablation could thus be of high value in the treatment of brain neoplasm. The recently published studies are limited and we therefore propose a safety and feasibility study for the use of cryoablation in patients with gliomas, meningiomas, and brain metastasis.

2. OBJECTIVES

This study aims to investigate the safety and feasibility of cryoablation in adults with gliomas (1. astrocytoma, IDH mutant 2. oligodendroglioma, IDH-mutant and 1p/19q-codeleted 3. glioblastoma, IDH-wildtype), meningiomas (WHO gr. 1 and gr 2), and brain metastasis.

Primary Objective:

The primary objective is to assess safety and feasibility in terms of:

- Safety: complications & morbidity
    - Descriptive: Postoperative intracranial bleeding, wound infection, epilepsy, brain edema, neurological deficit, death.
- Feasibility: Descriptive operation time, blood loss during intervention and practicability
- Technical failure

Secondary Objective(s):

The secondary objective is to analyze:

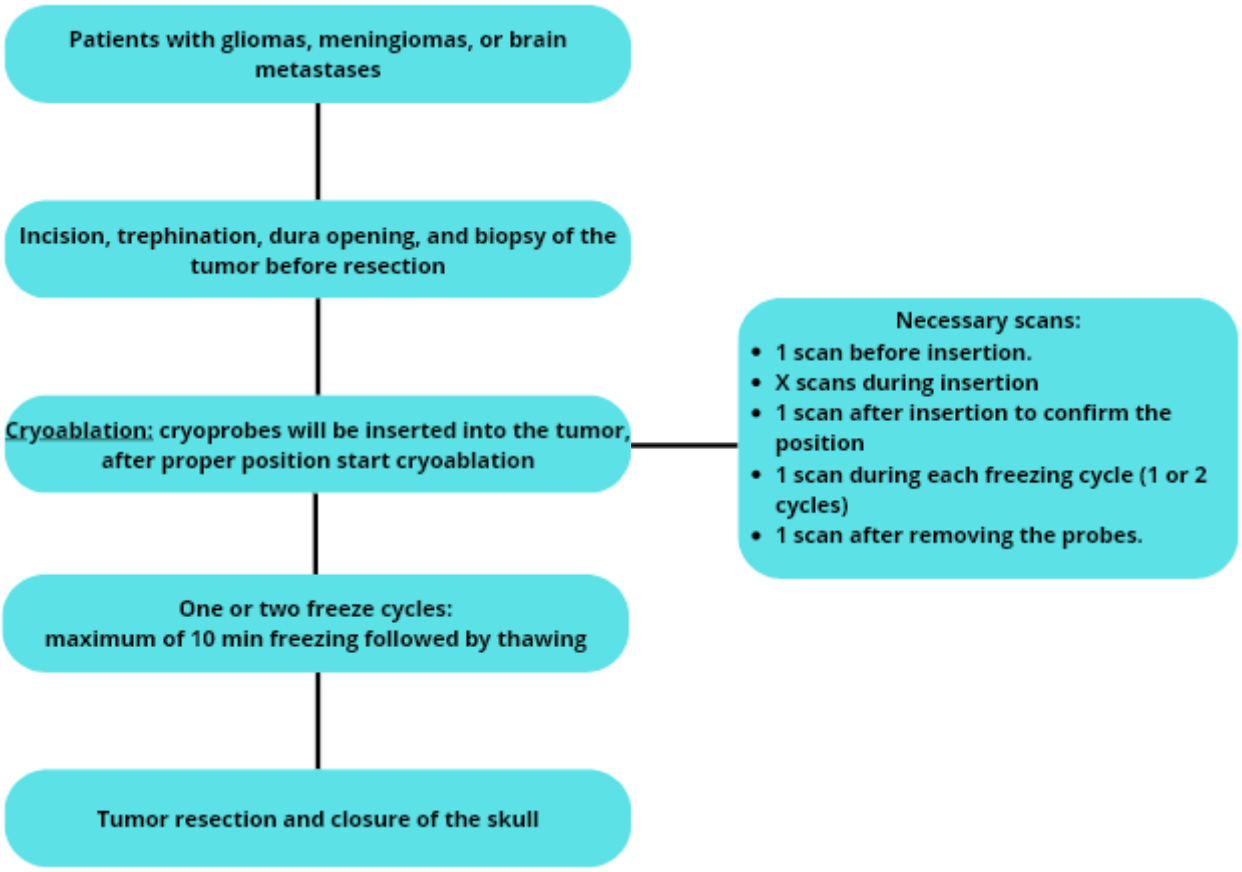
- The conformation of dead tumor cells by pathologist which have been cryoablated during surgery
- The confirmation of reaching macroscopic tumor edges using intraoperative – ultrasound (YES/NO). Pictures in 2 directions of the ultrasound will be made to objectify
- The progression free survival during the entire study period (3 months)
- Overall survival during the entire study period (3 months)
- Progression free survival and overall survival of the different tumors will be compared to matched historic controls.
- Safety and feasibility will be compared to historic matched controls

3. STUDY DESIGN

This study is an open label, prospective pilot study (Phase I study). Patients will be screened for inclusion and exclusion criteria, asked for consent, and when given, enrolled in the study. A total of 30 patients with gliomas, meningiomas, or brain metastasis will be included.

This study will include patients who are scheduled to undergo surgery for the resection of their brain tumor. All patients will undergo standard neurosurgery (skin incision, trephination, and dura opening) to expose cortex with underlying tumor or the tumor directly a vue. A biopsy will be taken for pathological diagnosis. After the establishment of the histopathological diagnosis, one or more cryoprobes will be inserted into the target tumor. Prior to cryoprobe placement, there will be one cone-beam CT-scan. The cone-beam CT is located in the operation room 2 where the operations will take place. The number of cryoprobes that are required depends upon the size of tumor. Proper positioning of the probes will be confirmed using cone-beam CT. During cryoprobe placement, a variable number of scans may be necessary. After proper positioning, one scan is needed to confirm the position and then cryoablation will start. The growth of the icecone will be monitored using intraoperative ultrasound and/or one cone-beam CT. The duration of each freezing cycle will be a maximum of 10 minutes, followed by active thawing. A second freezing phase (i.e. double freeze protocol) can be chosen if the surgeon deems it necessary. Additionally, there will be one scan after removing the cryoprobes. After removing the probes, the necrotic tumor will be resected. Tumor remnants will be further examined pathologically. Thereafter, the standard closure of the craniotomy will follow. Patients will receive the standard after treatment if necessary. Follow-up is up to 3 months after the treatment of the last included patient. A summary of the total study design is provided in **Figure 1.**

Figure 1- Overview of the study design



4. STUDY POPULATION

4.1 Population (base)

Patients will be recruited in the outpatient clinic. Adults with presumed gliomas (1. astrocytoma, IDH mutant 2. oligodendroglioma, IDH-mutant and 1p/19q-codeleted 3. glioblastoma, IDH-wildtype), meningiomas, or brain metastasis based on preoperative MRI or CT diagnosis will be asked for consent, and when given, enrolled in the study by the treating physician.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Age >18 years
2. Tumor suspected as glioma (1. Astrocytoma, IDH mutant 2. Oligodendroglioma, IDH-mutant and 1p/19q-codeleted 3. Glioblastoma, IDH-wildtype), meningioma (WHO gr. 1 and gr 2), or brain metastasis based on preliminary diagnosis for which the patient will undergo surgery
3. Supratentorial or infratentorial localization
4. Safe trajectory/trajectories possible for ablation of at least 70% of the tumor, avoiding eloquent structures
5. Karnofsky performance scale 70 or more
6. Sufficient knowledge of the Dutch language to understand the study documents (in the judgement of the attending physician or researcher)
7. Written Informed consent

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. <18 years or >80 years
2. Tumor diameter bigger than 10 cm
3. Unsafe trajectory (eloquent structures could be damaged)
4. Pregnancy
5. Contra-indication for general anesthesia

4.4 Sample size calculation

The total of 30 patients is based on the following: only very few clinical studies have been published on cryoablation of brain tumors. The study is therefore also explorative. The two most recent studies of gliomas included six and four patients respectively. (16, 17) The studies of meningiomas and brain metastasis had between 6 to 11 patients each. (18-20) Furthermore, there is little published guidance on sample sizes of safety and feasibility trials. According to Billingham et al. feasibility studies have a range of 10 to 300 patients per arm.(21) Based on other studies about cryoablation in neurosurgery and the audit of Billingham et al. we chose a sample size of 30 patients. We expect to include 30 patients within 12 months' time.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Treatment

On the evening before surgery 1.5–2.0 mg Lorazepam is administered for anxiolysis.
60 min. before anaesthesia induction the patient receives 1g Paracetamol p.o. and 7.5-15 mg midazolam p.o. if requested for sedation. 1g Cefazoline is given iv. for antibiotic prophylaxis before anaesthesia induction.
General anaesthesia is induced intravenously with fentanyl 0.25-0.5 mg, Propopfol 100-200 mg and cis-atracurium 10-20 mg. After induction of anaesthesia, patient is orotracheally intubated and mechanical ventilation is applied. Respiratory rate and tidal volume are adjusted to keep the patient normocapnic.
An arterial line, central venous catheter, and urinary catheter are inserted. Anaesthesia is maintained with Propofol (up to 10 mg/kg/h) and Remifentanil (0.5-2 µg/kg/min). Isoflurane (up to 1 MAC) and clonidine (1-2 µg/kg) may be added for maintenance, if necessary. The fluid management is aiming for normovolemia. 0.9% saline solution and balanced crystalloids are used for maintenance, in case of blood loss > 300 ml, HAES 130/0.4 solution will be given.
Temperature management is aiming for normothermia, warm-air blankets and warmed infusion lines are used. Arterial blood gas analysis is performed at the beginning of the procedure and repeated, if necessary. Electrolytes are controlled and substituted and hyperglycemia will be treated with insulin, if necessary.
The anesthetized patient is positioned on the table. Local infiltration of the scalp is performed with 20 ml Lidocaine 1% with Adrenaline 1:200.000 to reduce bleeding. The insertion points of the Mayfield Clamp are not infiltrated with local anaesthetics.

After surgical dissection (skin incision, trephination and dura opening) the tumor is exposed. Then a biopsy will be taken to confirm the diagnosis. After the establishment of the histopathological diagnosis, one or a number of cryoprobes required for tumor ablation will be inserted into the tumor. After proper positioning, the cryoablation will start. The ice formation will be monitored continuously, using cone-beam CT and/or intraoperative ultrasound. Prior to cryoprobe placement, there will be one scan. During cryoprobe placement, a variable number of scans may be necessary. After cryoprobe placement, there will be one scan to confirm the position. Additionally, there will be one scan during each freezing cycle (1 or 2 cycles), and one scan after removing the cryoprobes. The duration of each freezing cycle will be a maximum of 10 minutes, followed by active thawing. The final step is to resect the necrotic foci as a result of cryoablation guided by STEALTH-neuronavigation.

At the end of the procedure all anaesthetics are stopped and patient is brought to the Post Anaesthesia Care Unit (PACU). Detubation of the patient is performed as early as possible, if patient fulfils the detubation criteria (> 36 C body temperature, stable hemodynamics, sufficient spontaneous ventilation, adequate response to verbal orders). Postoperative analgesia is provided with Paracetamol i.v. or p.o. 1 g up to 4 dd and morphine 7.5 mg s.c. up to 4 dd, if necessary. At the PACU the patient is hemodynamically and neurologically monitored for 24 hours.

Post-intervention treatment:
Patients will receive the standard treatment as stated in the treatment protocols. There is no objection to radiation or chemotherapy after cryoablation

Products

Cryoablation device:
For this study, we will use the **ICEfx Cryoablation** System from the manufacturer Boston Scientific.

The ICEfx is the next generation of ablation systems with a compact design. The interface of the ICEfx Cryoablation System offers predictable and reliable ablation performance. This ablation system is intended for cryoablative destruction of tumor tissue during minimally invasive procedures. The ICEfx Cryoablation System is designed to destroy tumor tissue by the application of extremely cold temperatures. The system is able to reach temperatures of -40 °C using argon gas and can thaw the ice formation using helium gas.
This system is indicated to destroy prostate and kidney tissue, liver metastasis, tumors, and skin lesions. According to the manufacturer, the ICEfx Cryoablation device has the following indications:

- Urology Ablation of prostate tissue in cases of prostate cancer and Benign Prostate Hyperplasia (BPH)
- Oncology Ablation of cancerous or malignant tissue and benign tumors, and palliative intervention
- Dermatology Ablation or freezing of skin cancers and other cutaneous disorders, Destruction of warts or lesions, angiomas, sebaceous hyperplasia, basal cell tumors of the eyelid or canthus area, ulcerated basal cell tumors, dermatofibromas, small hemangiomas, mucocele cysts, multiple warts, plantar warts, actinic and seborrheic keratosis, cavernous hemangiomas, peri-anal condylomata, and palliation of tumors of the skin
- Gynecology Ablation of malignant neoplasia or benign dysplasia of the female genitalia
- General surgery Palliation of tumors of the rectum, anal fissures, pilonidal cysts, and recurrent cancerous lesions, ablation of breast fibroadenomas
- ENT Palliation of tumors of the oral cavity and ablation of leukoplakia of the mouth
- Thoracic surgery (with the exception of cardiac tissue)
- Proctology Ablation of benign or malignant growths of the anus or rectum

No specific contraindications are known for the use of ICEfx Cryoablation System. Similar systems have already been used in other (European) clinical studies and approved by the hospital ethics committees (16-20, 22, 23). Potential side effects that may be associated with cryoablation use can be organ-specific or general.

ICEfx uses five different kinds of needles; IceFORCE 2.1, IcePearl 2.1, IceRod 1.5, IceSeed 1.5, and IceSphere 1.5. Iceball shapes (ellipsoidal or spherical) and sizes are specific to the needle brand (**Figure 2**). There are also needles with different angles available.

The ice formation around the cryoprobes occur at different temperatures. The first layer around the needle is, with -40 °C the coldest, followed by the second layer with -20 °C. These two layers are mainly responsible for tumor tissue destruction. The outer layer is 0 °C.

The cryoprobes of this system are supplied sterilized and are not reusable.

Ultrasound:
We will use the BK Medical Ultrasound or Verasonics Vantage-256 functional ultrasound to position the cryoprobes and to monitor the formation of the Iceball.

Computer tomography:
Cone-beam Computer Tomography in operation room 2.

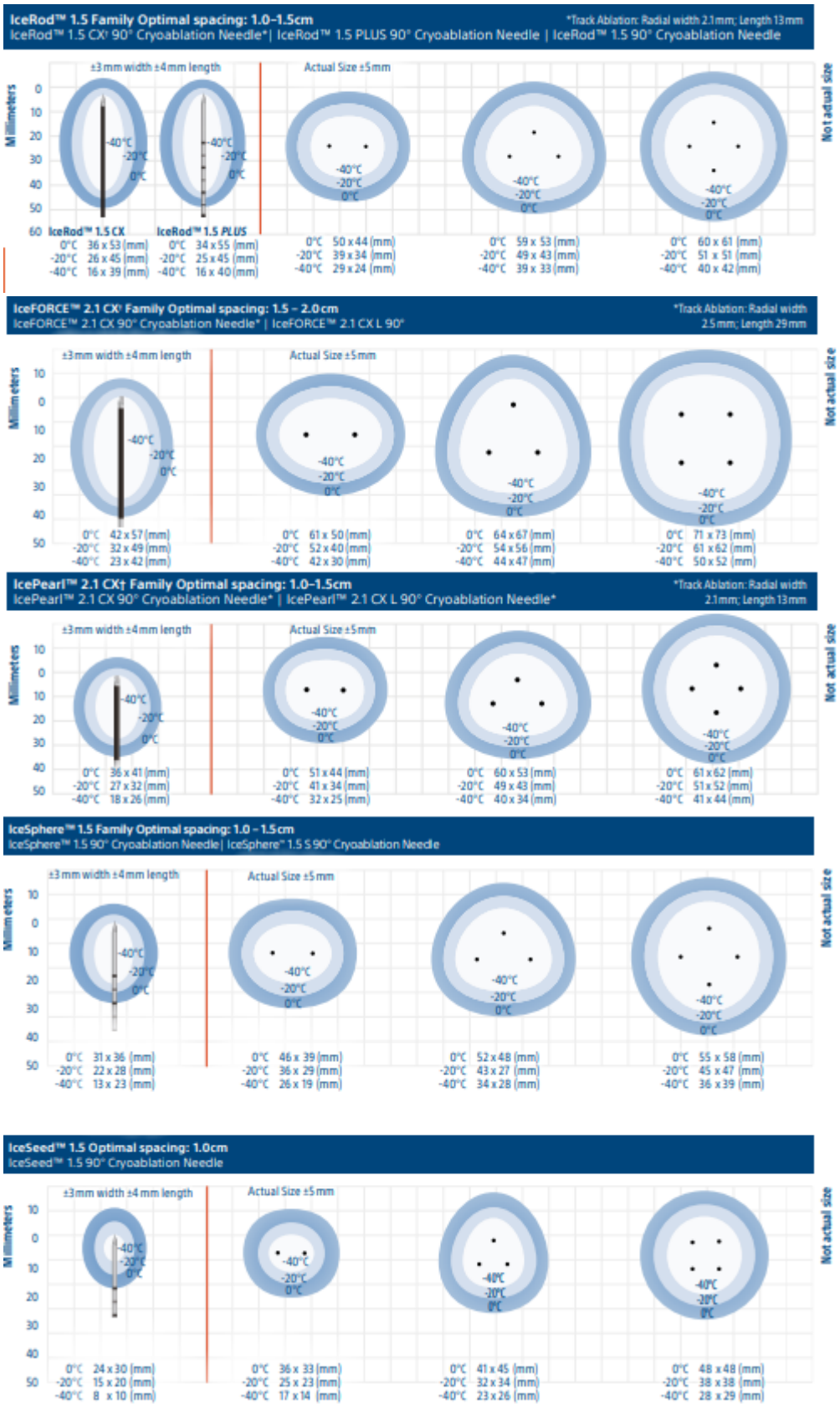
5.2 Use of co-intervention (if applicable)

Not applicable.

5.3 Escape medication (if applicable)

Not applicable.

Figure 2- Iceball shapes and sizes:



6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

For this study, we will use the **ICEfx Cryoablation** System from the manufacturer Boston Scientific. For more description see 5.1

6.2 Summary of findings from non-clinical studies

A total of 8 experimental studies used one of the investigational products or other similar products for ablation of tumors in glia cell model mice and rats. See **figure 3** for a global overview of these 8 studies (25-32).

In summary:

- Cryoablation increased the cellular immunity by activation of dendritic cells
- Cryoablation stimulate the production of specific antitumor antibodies and increase the activation of the immune system which may provoke an anti-tumoral response

6.3 Summary of findings from clinical studies

There are 7 clinical studies were similar products as ICEfx have been used to treat brain neoplasm. See **figure 4** for a global overview of these 7 studies (16-20, 22, 23).

6.4 Summary of known and potential risks and benefits

Although only few clinical cases have been published, cryoablation seems not to increase complications or mortality rates in brain surgery.

6.5 Description and justification of route of administration and dosage

Not applicable.

6.6 Dosages, dosage modifications and method of administration

Not applicable.

6.7 Preparation and labelling of Investigational Medicinal Product

The cryoprobes of this system are supplied sterilized and are not reusable.

6.8 Drug accountability

Not applicable.

<Please describe the procedures for the shipment, receipt, disposition, return and destruction of the investigational medicinal products.>

Figure 3- Global overview experimental studies

Articles	Groupe	Localization	Models cell line	Cryotherapy	Analyze	Effect	Commentary
Yin et al.	NA	/	C57BL/6 and BALB/c mice GL261 glioma	3 cycles (−140°C) 3 mm	DC80: DC86 IL6, IL1β, IL12	Increase Increase	DC maturation
Li et al.	4	Subcutaneous	Wistar rat C6 glioma	1 cycle (−140°C) 2 mm	DC3 DC4 DC4/DC8	Increase Increase	Enhanced cellular immunity
Zhang et al.	4	Subcutaneous	C57BL/6 mice BALB/c mice GL261 glioma	3 cycles −140°C 2 mm	Il10	Decrease	Improve anti-tumor effect via DC activation
Huang et al.	4 Combined with rhTNFα	Brain Cortex	Wistar rat C6 glioma	2 cycles Nitrogène −196°C 2 mm	PCNA	Decrease	Enhancing the inhibition of proliferation Induction of apoptosis
Liu et al.	2	Subcutaneous	C57 mice GL261 glioma	3 cycles 3 mm	Caspase 9 Caspase 8	Activation of intrinsic pathway Activation of extrinsic pathway	Induction of apoptosis
Li et al.	4 Combined with IL12	Subcutaneous	Wistar rats C6 Glioma	2 cycles −140°C	DC11+ DC86+ IFNλ DC marker	Increase Increase Increase	Boosted immune function via DC activation. Activation Th1-type immunity
Lin et al.	4 Combined with DC	Subcutaneous	C57BL/6 mice GL261 glioma	2 cycles −140°C 2 mm	DC3, DC3/DC4 IL12 DC2DC4/DC3DC8	Increase Increase Increase	Enhance systemic antitumor immunity
Xu et al.	4 combined with GM-CSF	Subcutaneous	C57BL/6 mice GL261 glioma	−140°C 2 mm	InterferonλDC8+	Increase Increase	Enhance activation of DCs

Figure 4- Global overview clinical studies

Authors	Year	Patients	Histology	Cryotherapy	Follow-up	Complications	survival
Martynov et al.	2018	88	Gliomas	**Awake**, 2 cycles (4-6 min freezing)	2-6 months	1,1% of mortality 11,4% complications (8% permanent)	GBM: 12.4 months Anaplastic astrocytoma: 46.9 months
Cebulla et al.	2021	6	Recurrent GBM	Single cycle (8-10 min freezing)	8.5-15,8 months	1 patient with transient paresis of elevator eyelid muscle	9 months [IQR: 7.5-15.3]
Gangi et al.	2020	4	Recurrent GBM	2 cycles(8-10 min freezing)	5-21 months	No complication observed	
Patil et al.	2021	3	1 macroadenoma 1 proclactinoma 1 recurrent craniopharyngioma	1 or 2 cycles (5-9 min freezing)		No complication observed	
Li et al.	2010	6	Metastasis	2 cycles(10 min freezing)		No neurological deficit	5 patients survived at 3 months
Endo et al.	1993	6	4hemangioblastomas 1cavernous angioma 1hemangiopericytoma	Single cycle		No complication observed	
Maroon et al.	1992	13	11 meningiomas 1 adenocarcinoma 1 invasive squamous cell carcinoma	1-3 cycles(2-4 min freezing)		No complications observed	

7. NON-INVESTIGATIONAL PRODUCT

<This chapter is applicable for any other product that is used in the study, like challenge agents or products used to assess end-points in the trial. This can be a medicinal product or a food product or a chemical compound or stable isotope or other product.
*This chapter does **not** include co-medication or escape medication, these are already mentioned in chapter 5*
For products to be used as in usual clinical practice the information can be limited to the chapters 7.1, 7.6 and 7.7 >

7.1 Name and description of non-investigational product(s)

7.2 Summary of findings from non-clinical studies

Not applicable.

<One may refer to the Investigator's Brochure (IB), Investigational Medicinal Product Dossier (IMPD), Summary of Product Characteristics (SPC) or a similar document (if applicable), by mentioning the relevant pages in that document. Be sure that the information is up to date and references to peer reviewed papers in (biomedical/scientific) journals should be given where appropriate.>

7.3 Summary of findings from clinical studies

Not applicable.

<See explanatory text of chapter 7.2, including remark>

7.4 Summary of known and potential risks and benefits

Not applicable.

<See explanatory text of chapter 7.2, including remark>

7.5 Description and justification of route of administration and dosage

Not applicable.

7.6 Dosages, dosage modifications and method of administration

Not applicable.

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable.

7.8 Drug accountability

Not applicable.

<Please describe the procedures for the shipment, receipt, disposition, return and destruction of the non-investigational medicinal products.>

8. METHODS

8.1 Study parameters/endpoints

The main objective is to assess the safety and feasibility of cryoablation use in patients with brain neoplasm.

8.1.1 Main study parameter/endpoint

Safety: complications & morbidity during the entire study period as decided by the treating physician. Severity and frequency of the following complications:

- Postoperative intracranial bleeding
- Wound infection
- Epilepsy
- Brain edema
- Neurological deficit (paresis/plegia)
- Aphasia
- Death

Feasibility:

- Operation time (in minutes)
- Blood loss during intervention (in milliliters)
- Practicability (questionnaire)

8.1.2 Secondary study parameters/endpoints (if applicable)

- The confirmation of reaching macroscopic tumor edges using intraoperative ultrasound (YES/NO). Pictures in 2 directions of the ultrasound will be made to objectify
- The conformation of dead tumor cells by pathologist which have been cryoablated during surgery
- Progression free survival at the end of the study, estimated at 3 months after surgery. The progression free survival is defined as time from diagnosis to disease progression (moment of progression as decided by a multidisciplinary team or by treating physician) or death, whichever comes first.
- Overall survival from intervention to end of study (3 months0.
- Progression free survival and overall survival of the different tumors will be compared to matched historic controls.
- Safety and feasibility will be compared to historic matched controls. Outcome will be compared using percentages after statistical analysis in the whole group.

8.1.3 Other study parameters (if applicable)

Not applicable

8.2 Randomisation, blinding and treatment allocation

Only patients who comply to inclusion and exclusion criteria are included after informed consent. There is no randomization.

8.3 Study procedures

Prior to operation:
New patients will be screened for inclusion and exclusion criteria, asked for consent, and when given, enrolled in the study. The patient will enroll in the study after the patient has signed the informed consent.

Intervention:
After the standard craniotomy, durotomy, and exposing the tumor as part of a surgical procedure, a biopsy will be taken to confirm the diagnosis. Under the guidance of ultrasound and/or cone-beam CT, a cryoprobe will be inserted into the tumor tissue. Multiple probes may be inserted if necessery. The number of cryprobes depends on the tumor size. After positioning the probes, cryoablation system will be set and ice formation will start. The ice formation will be monitored continuously. Prior to cryoprobe placement, there will be one scan. During cryoprobe placement, a variable number of scans may be necessary. After cryoprobe placement, there will be one scan to confirm the position. Additionally, there will be one scan during each freezing cycle (1 or 2 cycles), and one scan after removing the cryoprobes. The freeze cycle will take a maximum of 10 min followed by active thawing using the thawing mode of the cryoablation system. The central temperature of the iceball will be -40 °C using argon gas from the ICEfx Cryoablation system. After the freeze cycle, the necrotic tumor tissue will be resected. A summary of the total study procedure is provided in **Figure 1**. Lastly, the dura and the skull will be closed and the patient will receive standard postoperative care.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal (if applicable)

Not Applicable.

8.5 Replacement of individual subjects after withdrawal

Included patients who underwent cryoablation will not be replaced. When a patient is withdrawn by the investigator or withdrawn him/herself before intervention, this patient will be replaced if a next eligible patient is available.

8.6 Follow-up of subjects withdrawn from treatment

There will be no follow up of patients withdrawn from the study. Patients will be treated for his/her medical condition outside the study.

8.7 Premature termination of the study

The study will be terminated:

- In case of a very slow inclusion rate (<1 patients per month)
- In case of serious adverse events during the intervention
- In case of SAEs exceed 50% after every fifth patient is treated
- At the request of the sponsor

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.
A safety monitoring review will be performed after 5/10/15/20/25 patients have been treated and followed for one month after the procedure. The higher boundary for the SAEs rate is 50% of patients having experienced at least one SAE related to the cryoablation treatment procedure.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events. All adverse events will also be reported to Boston Scientific via E-mail

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

Not Applicable.

10. STATISTICAL ANALYSIS

We will perform a chi-square test of difference to test whether cryoablation-related complications (postoperative intracranial bleeding, wound infection, epilepsy, brain edema, neurological deficit, and aphasia) and blood loss during the intervention are related to historical matched controls. Normally distributed variables will be summarized using means and standard deviations and categorical variables will be summarized using percentages. For the primary and the secondary study outcomes, only the available data will be analyzed (no imputation of missing data). Only a p-value of <0.05 will be considered to be statistically significant.

Statistical analysis will be performed in all 30 patients together to prevent the statistical power from getting low.

10.1 Primary study parameter(s)

Primary study parameters are safety and feasibility. Safety will be expressed in terms of severity and frequency of the following complications: postoperative intracranial bleeding, wound infection, epilepsy, brain edema, neurological deficit, and aphasia. All data will be taken from the patients' file. The feasibility will be expressed in terms of operation time (in minutes), blood loss during the intervention (in milliliters), and practicability. Operation time and blood loss will also be taken from patient's file. For practicality, the neurosurgeons performing the cryoablation will be asked fill in a questionnaire, answering questions regarding their experiences with cryoablation. The main study parameters will be analysed at the end of the study and it will be based on the intention to treat principle.

10.2 Secondary study parameter(s)

The three secondary study parameters are the confirmation of reaching macroscopic tumor edges using intraoperative ultrasound, progression free survival, and the overall survival at the end of the study. Progression free survival and overall survival will be analysed by the Kaplan Meyer method.

10.3 Other study parameters

Not applicable.

10.4 Interim analysis (if applicable)

No interim analysis will be carried out. The researchers do not expect serious morbidity or mortality based on literature, and therefore will also not include a stopping rule.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. The project leader is responsible for the proper conduct of the study.

11.2 Recruitment and consent

The attending neurosurgeons will inform patients that may qualify for cryoablation on the opportunity to participate in this study. The patient will receive a patient information letter, general practitioner letter, and informed consent letter. Patients will have 4 days to think about participating and if necessary more in consultation with the physician or researchers.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

11.4 Benefits and risks assessment, group relatedness

Cryoablation is used in several medical specialties, often with better results than conservative therapies. Cryoablation offers precise and safe lesion targeting, is minimally invasive and the ice-cone formation within the tumor can be monitored by several imaging techniques. Cryoablation could also potentially be used for non-resectable tumors. Furthermore, cryoablation could have a potential immunological effect in some malignant brain tumors. Although very few clinical studies have been published, cryoablation seems not to increase complications or mortality rates for brain surgery. Cryoablation could thus be of high value in the minimal invasive treatment of several brain tumors.
Regarding the use of cone-beam CT: by taking additional scans, the placement of cryoprobes can be even more precise, increasing the possibility of achieving a larger ablation zone. Cone-beam CT is also more suitable for deeper-seated tumors compared to ultrasound, making it more feasible in the future. Additionally, cryoablation is often performed with the assistance of cone-beam CT in other pathologies. Considering the age (often 60+) and limited life expectancy of a significant portion of the patients (including glioma patients and patients with brain metastases), it can be assumed that the radiation burden is acceptable.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives (if applicable)

Not applicable.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

The handling of personal data will comply with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation. (in Dutch: Uitvoeringswet AVG, UAVG).. Data of each patient will be coded by a unique patient study number at enrolment. All data will be collected, stored and locked up in a cabinet by the investigational team. Only the investigators will have access to all patient information.

The data will be transferred to a central database by the investigators, which will be protected by a password only known to the principal investigator. The backup procedure will be followed weakly.

12.2 Monitoring and Quality Assurance

Monitoring will be done according to monitoring plan B, “Onderzoek met een matig risico”.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject,

numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as 4 weeks after the intervention of the last patient.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

This clinical study will be registered in the Dutch Trail Register before the first patients are recruited. The results of the study will be published in the international literature and made available to patients who require results.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable.

a. Level of knowledge about mechanism of action
b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism
c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?
d. Selectivity of the mechanism to target tissue in animals and/or human beings
e. Analysis of potential effect
f. Pharmacokinetic considerations
g. Study population
h. Interaction with other products
i. Predictability of effect
j. Can effects be managed?

13.2 Synthesis

The cryoablation devices that we will use will be used as intended to use. At the start of the study, the attending neurosurgeons who will perform the operation will be well trained by the manufacturer. All patients included will be operated on as standard treatment followed by adjuvant therapy. Exclusively the freezing cycles preceding tumor removal and the supplementary CT scans will be conducted for the included patients. The application of cone-beam CT is proportionate to the condition, age, and average prognosis of the included patients. The suitability of cone-beam CT for deeper structures justifies its utilization, as this enhances its feasibility for potential future treatments of these conditions and facilitates future research endeavors. The complications and mortality of cryoablation are similar to ordinary resection.

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