Protocol for the HYPER-study

Contents

1. Title

English: Hyperexcitability as a Predictor of Epilepsy Risk in Glioblastoma (HYPER-study)

Danish: Hyperexcitabilitet til forudsigelse af epilepsi hos patienter med glioblastom

2. Aim

a. Problem statement, hypothesis, endpoints, and rationale:

Recent advances in research on brain tumors, called gliomas, have improved our understanding of its molecular biology but have not significantly enhanced survival or quality of life. Evidence suggests that neuron-glioma interactions drive tumor growth, disrupting brain networks and causing epileptic seizures, leading to worse outcomes. However, not all tumors follow this pathway, and we cannot yet predict which patients will develop seizures. By integrating clinical data, genomics, and real-time brain activity measurements, our ambition is to identify patients at high risk of developing seizures.

By highlighting three different aspects of epilepsy (diagnosis, seizures, and medication), we can obtain valuable data to support or disprove our hypothesis about epilepsy's negative impact on brain cancer. The results can be used to design clinical trials aiming at improved personalized treatment.

Our hypothesis is that epilepsy-related high-grade gliomas represent a clinical and molecular subgroup of gliomas with increased synaptic activity that drives tumor growth thereby causing a poorer prognosis for the patients.

The aim of the HYPER study is to investigate how hyperexcitability is related to development of epilepsy, seizure control, and survival in patients with glioblastoma.

Hyperexcitability will be measured using subcutaneous EEG.

The endpoints for this study are development of epilepsy, seizure control, overall survival, and progression-free survival.

The HYPER study is the first to investigate hyperexcitability as a potential early driver of tumor progression in glioblastoma patients. If hyperexcitability is shown to contribute to tumor growth, future clinical trials should focus on targeting hyperexcitability to achieve tumor control. The overarching goal is to enhance overall survival.

b. Short review of literature:

Glioblastoma is the most common and aggressive primary brain tumor in adults[1]. Treatment consists of maximal safe resection, radiotherapy, and chemotherapy. Despite this the median overall survival for glioblastoma is only 15 months[2].

Epilepsy is one of the most common symptoms in gliomas[3-5]. Glioma-related epilepsy (GRE) negatively impacts quality of life[6]. GRE treatment with anti-seizure medication (ASM) is given following the first epileptic seizure to achieve durable seizure control and improved quality of life.

In patients with epilepsy, the neurons are both firing too much and too synchronized. Hyperexcitability is a term used in this area of research to describe the excessive firing of neurons. Preclinical studies show that excessive firing between glioma cells and neurons drive glioma growth and invasiveness and that treatment with ASM inhibits tumor growth[7,8]. A recent large epidemiological study found that patients with epileptic seizure after glioma diagnosis had a significantly poor prognosis[9]. Taken together these studies suggest hyperexcitability and GRE drive glioma growth and negatively impact survival in HGG patients.

In clinical practice, hyperexcitability can be measured with electrodes placed on the scalp called electroencephalogram (EEG). An EEG from a patient with epilepsy can reveal specific hyperexcitable electric patterns described as interictal epileptic discharges (IEDs)[10]. In contrast to the standard 30-minute scalpEEG, a novel device has been developed by a Danish company (UNEEG) to continuously monitor electrical activity using electrodes under the skin (subcutaneous EEG (scEEG))[11]. The device is CE marked and we will use it as an instrument to continuous, long-term, EEG monitoring to improve the detection of IEDs and seizure activity compared to routine 30-minute EEG. Routine EEG often fails to capture transient abnormalities due to its short duration, leading to missed diagnoses. In contrast, scEEG enables prolonged recording, increasing sensitivity to rare events and providing a more comprehensive assessment of seizure patterns. Additionally, scEEG improves diagnostic accuracy in

patients with infrequent seizures, allowing for treatment optimization. Its minimally invasive nature makes it suitable for outpatient settings, enhancing patient comfort and compliance while reducing hospitalization needs. Thus, this device enables continuous investigation of hyperexcitability as part of epilepsy and tumor development – this causal relationship has only been studied in laboratory experiments. Knowledge from patients could confirm and shed light on this disease mechanism, potentially leading to better treatment. A video about the scEEG can be found via the QR code, or via this link: https://vimeo.com/1006556953?share=copy.

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3. Methods

a. Design, analysis method as well as the use of a possible control group, randomization, and placebo

The HYPER study is an observational cohort study. There will be no control group, randomization, or use of placebo.

Hyperexcitability

To measure neuronal hyperexcitability in real-life settings, patients will undergo a scEEG.

Neuronal hyperexcitability will be defined as the presence of IEDs on the scEEG.

The scEEG will be inserted in the period from end of radiotherapy till first MRI evaluation after two cycles of adjuvant chemotherapy (Figure “Study flow”). Recordings from the device will start after implantation.

A specialized AI analytic software for managing long term EEG data will be used to highlight suspected IEDs that can be reviewed by a clinical neurophysiologist to determine if these episodes show epileptiform discharges[11,12].

Epilepsy

All patients who develop epilepsy will be treated at Rigshospitalet by Lars Pinborg, clinical professor in neurology with a special focus on epilepsy. Patients will be treated with standard treatment for epilepsy according to national guidelines[13].

b. The practical implementation, examinations, and scope

Patients will be informed and included via the Department of Cancer Treatment at Rigshospitalet. Detailed inclusion process is described in section 13. The doctors and nurses at the department are experienced in including patients for clinical trials.

A neurosurgeon (Rune Rasmussen, neurosurgeon at the Department of Neurosurgery, Rigshospitalet) will place a scEEG device under the skin behind the patient’s ear closest to the tumor. The device is inserted under local anesthesia in 20 minutes. The electrodes can be inserted with different directions and will be positioned closest to the tumor.

The scEEG will be explanted after fifteen months with the possibility of extension in case the scEEG device is approved for longer implantation. The scEEG does not need to be removed before cremation.

Patients will have an extra visit at Rigshospitalet when the scEEG is implanted and one when it is explanted. Patients who develop epilepsy might have extra visits at Rigshospitalet in relation to treatment with ASM. Otherwise, there will not be extra visits needed for participation in the study.

c. Deviations from standard treatment

There will be no deviations from standard treatment both regarding tumor and epilepsy treatment.

4. Statistical considerations

a. Power analysis

This study is explorative, and no studies have used scEEG to identify IEDs in brain tumor patients before. Therefore, making assumptions for power analysis is challenging since no data is available from similar study designs.

Our endpoints are development of epilepsy, seizure control, progression free survival and overall survival.

Based on current data from our clinical database the number of patients needed for this study is estimated to be fourteen patients. The level of significance is 2.5% one sided and the power 80%. The sample size is determined under the following assumptions: the proportion of patients having epilepsy is 50%, and the sensitivity for epilepsy with hyperexcitability is 85% or better. The null hypothesis is diagnostic accuracy = 0.5 against the alternative that diagnostic accuracy > 0.5.

5. The study participants

a. Inclusion criteria:

- Patients who have consented to the Neurogenom protocol[14]
- Patients 18 years of age or older
- Patients diagnosed with glioblastoma, IDH wild type grade 4
- Patients treated with standard therapy at Rigshospitalet, Department of Cancer Treatment
- Patient with a tumor located in a region where scEEG is expected to provide adequate signal quality
- Performance status 0-1
- Patients willing and able to use the scEEG, UNEEG EpiSight solution, day and night for the duration of the study
- Patients willing and able to provide written informed consent
- Patients able to understand the consequences of participation
- Patients able to complete all study-required procedures, assessments and follow-up

b. Exclusion criteria:

- Patients who have a diagnosis of epilepsy
- Patients treated with ASM
- Pregnancy
- High risk of surgical complications as assessed by the neurosurgeon
- Involved in therapies with medical devices that deliver electrical energy into the area around the implant, such as cochlear implant(s), implantable brain stimulation and external/transcranial brain stimulation
- Contraindications to the local anesthetic used during implantation and explantation

6. Risks, side effects, and disadvantages in the short and long term.

a. Safety measures that minimize pain, discomfort, fear, and other risks

Local anesthesia is used for implantation to minimize pain and discomfort under the procedure. As with any type of surgery there is risk of bleeding, infection, and nerve damage. After implantation there is a risk of displacement of the scEEG and irritation/inflammation around the implant. Patients will receive thorough information on signs of inflammation and instructions on how to wash the area behind the ear to minimize the risk of irritation and infection. Also, the patient will be provided with contact information of the Department of Neurosurgery who they can contact directly at any time of the day if they have concerns about the implant. The patients will be given contact information of the principal investigator in case of other study related concerns.

To date about two hundred people has had the scEEG implanted. No systematic collection of side effects is available concerning the scEEG. The estimated infection rate is 2-4 %. In the UK, one incidence of skin penetration due to surgeon error during the implantation has been reported.

b. Risk of radioactive radiation

There is no risk of radioactive radiation

7. Collection of new biological material or retrieval of biological material from an existing biobank

For the HYPER study no new biological material will be collected.

8. Information from patients’ records

a. Disclosure <u>without consent</u>

Describe:

i. Whether it is necessary to disclose information to identify/recruit study participants:

It is not necessary to share information with others about the patients to identify them. They will all be treated at the Department of Cancer Treatment at Rigshospitalet.

ii. Whether an exemption from obtaining consent is being sought or whether data from the patient record will be included without consent:

There will be no exemptions from obtaining consent and no data will be included without consent.

iii. Whether a retrospective control group will be included:

No retrospective control group will be included

iv. For all categories it must be described:

a. What specific information that need to be disclosed

Not applicable in this context.

b. The purpose
   Not applicable in this context.
c. How many patient records it concerns
   Not applicable in this context.
d. What time period the information from the patient records is from
   Not applicable in this context.

v. It must be stated that the information to be used in the project is disclosed to the researcher before consent has been given by the study participants
   Only the doctors at the Department of Cancer Treatment will have access to the patients records before consent has been given. Information before consent is not shared with others.

b. Use of patient record information <u>with consent</u> (after inclusion)
   Describe:
   i. Which specific information will be collected for the trial
      Listed below

   ii. The purpose (how they contribute to clarifying the projects hypotheses)

Category	**Purpose**
Name	For identification
CPR number	
Diagnosis	To show characteristics of the cohort
Pathology report	
Performance status	
MRI	
Medication	
New symptoms (e.g., paralysis)	For correlation with hyperexcitability
Seizures	
ASM dosage and start/end dates	
Date of progression	
Date of death	
Side effect to ASM	For ASM treatment adjustments
Side effect of the implant	To monitor if explantation is needed

   iii. It must be stated that the consent grants the trial responsible, sponsor, and sponsor's representatives, as well as any regulatory authority, direct access to obtain information from the patient's medical records, including electronic records. This access is intended to review information about the participant's health conditions necessary for conducting the research project and for control purposes, including self-monitoring, quality control, and monitoring, which they are obligated to perform.
      This information will be given to the patients before inclusion: Consent grants the trial responsible, sponsor, and sponsor's representatives, as well as any regulatory authority, *direct* access to obtain information from the patient's medical records, including electronic records. This access is intended to review information about the participant's health conditions necessary for conducting

the research project and for control purposes, including self-monitoring, quality control, and monitoring, which they are obligated to perform

9. Handling of personal data in the project

a. You must state that “databeskyttelsesforordningen” and “databeskyttelsesloven” are being complied with

“Databeskyttelsesforordningen” and “databeskyttelsesloven” are being complied with, and patients will get information about this with the written consent.
The project will be registered in the Capital Regions research database Privacy.

10. Transmission of personal data/biological material abroad

Data will not be sent abroad.

11. Economy

a. Describe who has initiated the trial

The principal investigator (Maria Dencker) and her supervisors (Thomas Urup and Lars Pinborg) have initiated this study.

b. Name of sponsors, including the amount for each sponsor.

This project is sponsored by Krogh Invest ApS. Total amount: 3.300.000 DKK
Replies on applications for other sponsors to cover cost of the scEEG are awaiting. The committee and the study participants will be informed of any funding received as it becomes available.

c. How the support is incorporated into the trial

Krogh Invest ApS is sponsoring salary (full time for Maria Dencker, part time for Thomas Urup, part time for statistician) and operating cost.

Budget

Kategori	**Beskrivelse**	**Beløb i DKK**
Videnskabelig arbejdskraft	Løn, PhD-studerende, 36 måneder	2.100.000
Videnskabelig arbejdskraft	Løn, Postdoc, 12 måneder	700.000
Videnskabelig arbejdskraft	Løn, statistiker, 2 måneder	100.000
Udstyr	scEEG’er	1.200.000
Drift	Dataindsamling, publikationsudgifter mm	300.000
	Total	4.500.000

d. Whether the support is paid directly to the researcher, their department/institute, a research fund, or another entity.

The support is paid to a research account managed by the Department of Cancer Treatment.

e. Whether the researcher has financial ties to the sponsor or other stakeholders in the trial

None of the researchers have financial ties to the sponsor

12. Any compensation and/or other benefits for the trial participants.

The participating patients do not receive any compensation.

13. Recruitment of trial participants and informed consent

Describe the recruitment process as well as the procedure for providing oral information and obtaining consent:

a. How the trial participants are found and identified

Patients will be identified through visits to the Department of Cancer Treatment at Rigshospitalet.

b. How the initial contact with the trial participant takes place

Patients have regular visits at the hospital during the six weeks of radio-/chemotherapy. During these visits, patients will be made aware about the study by nurses or doctors at the Department of Cancer Treatment. If the patient is interested in participation, they will be referred to the principal investigator or to one of the doctors who have received training in patient enrollment and have consequently been delegated the responsibility for including patients in the study. The trial participant and the principal investigator or delegated doctor will then meet at the Department of Cancer Treatment, and the trial participant will be informed about the study.

c. Process of obtaining informed consent

i. Where, when, and who provides the oral and written information

The patient will receive written and oral information about the study either by the principal investigator or the delegated doctor. This visit is voluntary and will take place at the Department of Cancer Treatment.

All relevant doctors at the department will be trained in patient enrollment and consequently be delegated the responsibility for including patients in the study. The delegation log will be attached to this application.

The written and oral information will be given in the period from start of radio-/chemotherapy till first MRI evaluation after radio-/chemotherapy. See attached figure “Study flow”.

ii. How it is ensured that the discussion takes place undisturbed

Consultations with patients and their relatives will be in a private and undisturbed room during their visit to the Department of Cancer Treatment.

iii. How the right to a companion is ensured

The nurses and doctors at the department will tell the patients about the right to an accompanying person before the information meeting with the principal investigator or delegated doctor.

iv. The reflection period between receiving oral and written information and providing informed consent

The patients will have a right for >24 hours of reflection time.

v. When consent is sought

Consent is obtained once the patient has had sufficient time to reflect. Consent can be given to either the principal investigator or delegated doctor. Consent can be given eighter at the next scheduled visit to the department or at an extra visit.

14. Publication of results

Both positive, negative, and inconclusive results will be published in international peer-reviewed scientific journals. If publication is not achieved, the results will be disseminated through alternative methods, such as presentation at conferences.

15. Ethical considerations

The study will be conducted in accordance with ethical guidelines for medical research involving human subjects, as outlined in the Declaration of Helsinki.
Participation in the study is entirely voluntary, and patients can withdraw at any time without consequences for their standard treatment. If a patient decides to withdraw, their data will be removed from the study unless they consent to continued use.

Describe:

a. Why the risks are not, in themselves or in relation to the benefits of the trial, irresponsible

As described in section 6 only one side effect directly associated with the procedure has been reported out of two hundred procedures. Experiences from previous implantations have helped minimize the risk of errors in upcoming procedures.

b. Why the therapeutic benefit for the trial participants or future patients justifies the study

Based on the very modest risks and inconveniences, as well as the therapeutic benefits for future patients through more effective and targeted treatment, it is considered scientifically and ethically justifiable and warranted to conduct this project.

16. Information about insurance

a. Whether the trial is covered by the patient compensation scheme or if a separate insurance policy is taken out.

The trial is covered by the patient compensation scheme (Patienterstatningen).