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Whole Neuraxis Low Dose Radiotherapy (LDRT) in patients with Amiotrophic Lateral Sclerosis (ALS)

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Deutsche Zusammenfassung

Die amyotrophe Lateralsklerose (ALS) ist eine unheilbare Krankheit und alle Medikamente und sonstigen Maßnahmen sollen dazu beitragen, die Muskelfunktion und Lebensqualität möglichst lange aufrechtzuerhalten. Im Laufe der Zeit ist jedoch mit einer weiteren Verschlechterung der Muskelfunktion zu rechnen.

Die Strahlentherapie ist heute ein wichtiger Bestandteil der Behandlung von Krebspatienten. Diese Patienten erhalten in der Regel hohe Strahlendosen - z. B. eine Mindestdosis von 1,8 Gy, und sie werden mehrere Wochen lang täglich bestrahlt.

Die niedrigdosis-Strahlentherapie ist eine therapeutische Maßnahme, die auf verschiedene Zellen des Immunsystems einwirkt und dessen Wirkmechanismus moduliert. Eines der wichtigsten therapeutischen Ziele der niedrigdosis-Strahlentherapie ist die Entzündung, und aufgrund dieser Eigenschaft wird die aktiv bei der Behandlung von Patienten mit verschiedenen entzündlichen und degenerativen Gelenkerkrankungen eingesetzt, insbesondere bei solchen, bei denen andere Maßnahmen nicht wirksam sind.

Diese therapeutische Intervention wurde noch nie bei Patienten mit ALS eingesetzt. In ersten klinischen Studien, bei Patienten mit der Alzheimer-Krankheit, die ebenfalls eine neurodegenerative Erkrankung ist, hatte die Behandlung mit einer niedrig dosierten Strahlentherapie vielversprechende Ergebnisse erzielt, und die einzige Nebenwirkung war ein vorübergehender Haarausfall mit zufriedenstellendem Haarwachstum. Aus diesem Grund wurden weltweit mehrere klinische Studien mit denselben Patienten eingeleitet.

Die Entzündung spielt auch bei ALS eine wichtige Rolle, und wir vermuten, dass durch die Verringerung der Entzündung auch positive Auswirkungen zu erwarten sind.

Um diese Hypothese zu beweisen, werden wir eine einarmige, unkontrollierte Pilotstudie mit 5 Patienten mit ALS durchführen. Geplant ist die Bestrahlung der gesamten Neuroachse (Gehirn und Wirbelsäule) der Patienten mit einer Gesamtdosis von 1 Gy in 5 täglichen Fraktionen (Einzeldosis 0,2 Gy). Wir haben uns für dieses Dosierungsschema entschieden, weil es bei der Behandlung anderer degenerativer und entzündlicher Erkrankungen keine klare Dosis-Wirkungs-Beziehung gibt und weil die Gesamtdosis von 1 Gy keine Nebenwirkungen verursachen sollte. Wenn sich dieses Konzept als praktikabel erweist und erste relevante Behandlungseffekte zu beobachten sind, planen wir eine randomisierte Studie

Scientific Background

New effective treatment options for amyotrophic lateral sclerosis (ALS) are an absolutely unmet need. Despite advances in molecular biology and genetics, our understanding of this disease remains limited [1]. Oxidative stress is one of the factors causing the loss of motor neurons (MNs) and mitochondrial dysfunction in patients with ALS [2]. Neuroinflammation also plays a crucial role in the pathogenesis of ALS and is characterized by infiltration of lymphocytes and macrophages, activation of microglia and reactive astrocytes, as well as the involvement of complement [2]. All these alterations, directly or indirectly, have an impact on MN degeneration [3].

Low Dose Radiotherapy (LDRT) modulate immune response acting on different cells (plasmacytoid dendritic cells, B-cells, monocytes) involved in this complex process [4, 10]. Due to these properties, LDRT has been actively used in the treatment of patients with various inflammatory and degenerative joint diseases. The recommended total dose for painful joint disease is usually between 3-6 Gy, administered in 0.5-1 Gy per fraction. On the other hand, a randomised clinical trial has shown that the effects of LDRT are not dose-dependent (no statistical difference between a total dose of 3 Gy and 0.3 Gy) [5].

The neurobiological effects and mechanisms of the brain's response to LDRT are not well understood. Animal experiments demonstrated that LDRT can modulate the phenotype of microglia cells, induce repair mechanisms, mitigate oxidative stress, stimulate defences against neuroinflammation and stimulate neural stem cell proliferation promoting neurogenesis in the hippocampus [7]. It has been shown that in AD mouse model treatment with LDRT modulates microglia phenotype by promoting M2 polarization [11].

Animal and human studies with neural stem cell cultures irradiated at 0.05–0.25 Gy showed increased levels of ATP and decreased ROS/RNS levels, which contribute to increased cell survival, as opposed to cells irradiated at higher doses of 1 Gy [7].

Due to the immunomodulatory and anti-neuroinflammatory properties of LDRT, numerous clinical trials have been initiated worldwide to investigate the effects of LDRT in patients with AD. (Home — ClinicalTrials.gov) - NCT03597360, NCT02769000, NCT02359864, NCT04203121, NCT05635968, NCT03352258). In one pilot clinical trial, five patients with AD were treated with whole brain radiotherapy (10 Gy in 5 fractions, single dose 2 Gy) [8]. The Mini-Mental State Examination (second edition) T-scores at 1 year mark showed the positive changes in three patients, while one remained stable. The post-treatment scores of three improving patients increased to the average range. No safety issues were reported, and the only side effect was temporary alopecia with satisfactory hair regrowth.

Hypothesis and Aims

We anticipate that patients with ALS will also benefit from LDRT. The immunomodulation and reduction of neuroinflammation from this procedure will lead to an improvement in axonal transport and synaptic function, resulting in improved functionality and quality of life.

To prove this hypothesis, we propose a single-arm, uncontrolled pilot study involving 5 patients with ALS. Our plan is to irradiate the whole neuroaxis of the patients with a total dose of 1 Gy in 5 daily fractions (single dose 0.2 Gy). We have chosen this dose regimen because there is no clear dose-response relationship in the treatment of other degenerative and inflammatory diseases [6] and because the total dose of 1 Gy should

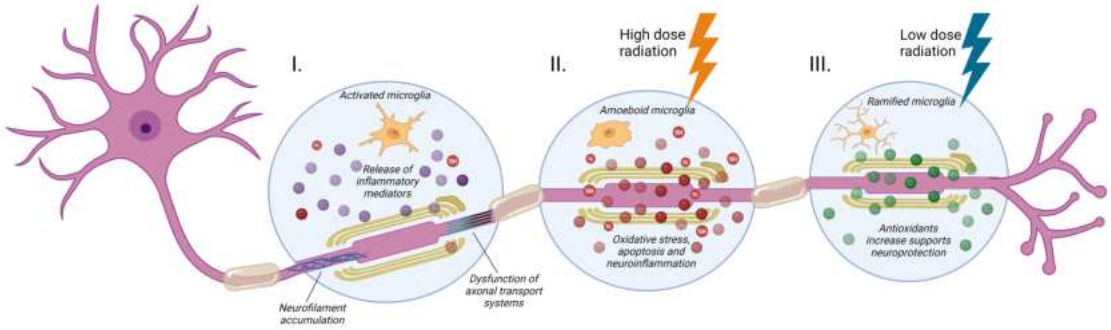

Figure 1: Pathophysiology of ALS and supposed influence of high and low dose radiation [7,12]. Created with BioRender.com.
I. Microglia activation results in secretion of proinflammatory cytokines and neurotoxicity, leading to neuronal damage.
II. High dose ionising radiation provokes a neuroinflammatory response via amoeboid, pro-inflammatory cytokines and reactive oxygen species which can have deleterious effects on cell functioning and survival.
III. Low dose ionizing radiation may confer neuroprotection by decreasing neuroinflammation, increasing antioxidant levels and reducing oxidative stress.

not cause any side effects. If this concept proves feasible and the first signs of treatment success are seen, we plan to conduct a randomised trial.

Methods and Design

In order to prove our hypothesis, we propose to conduct following measurements

Material: CSF
We will use cerebrospinal fluid obtained at the time of diagnosis confirmation and 4 weeks after completion of treatment with LDRT

- We expect neuroinflammation to be reduced after treatment with LDRT compared to baseline. We will use a standard neuroinflammation kit to measure the expected changes in neuroinflammation markers (Uni Düsseldorf).
- We assume that oxidative stress would decrease after treatment with LDRT compared to baseline. Therefore, we propose that the 8-OHdG concentration (marker for oxidative stress) in CSF and urine will decrease after treatment with LDRT. For this purpose, we will use an 8-OHdG ELISA kit. (Thermo Fischer).
- We hypothesize that neuronal damage will be reduced after treatment with LDRT. To confirm this, we will measure NfL levels in CSF and p75ECD levels in urine before and after treatment with LDRT using commercially available kits.

Method: ALS-FRS-R
We will use ALS-FRS-R obtained at the time of diagnosis confirmation and 4 weeks after completion of treatment with LDRT

- We supposed that the motoric function of patients with ALS can be improved or at least remain stable. For this purpose, we will measure ALS-FRS-R score.

Other Examinations
Baseline and 2nd post-treatment (4 weeks after completion of treatment with

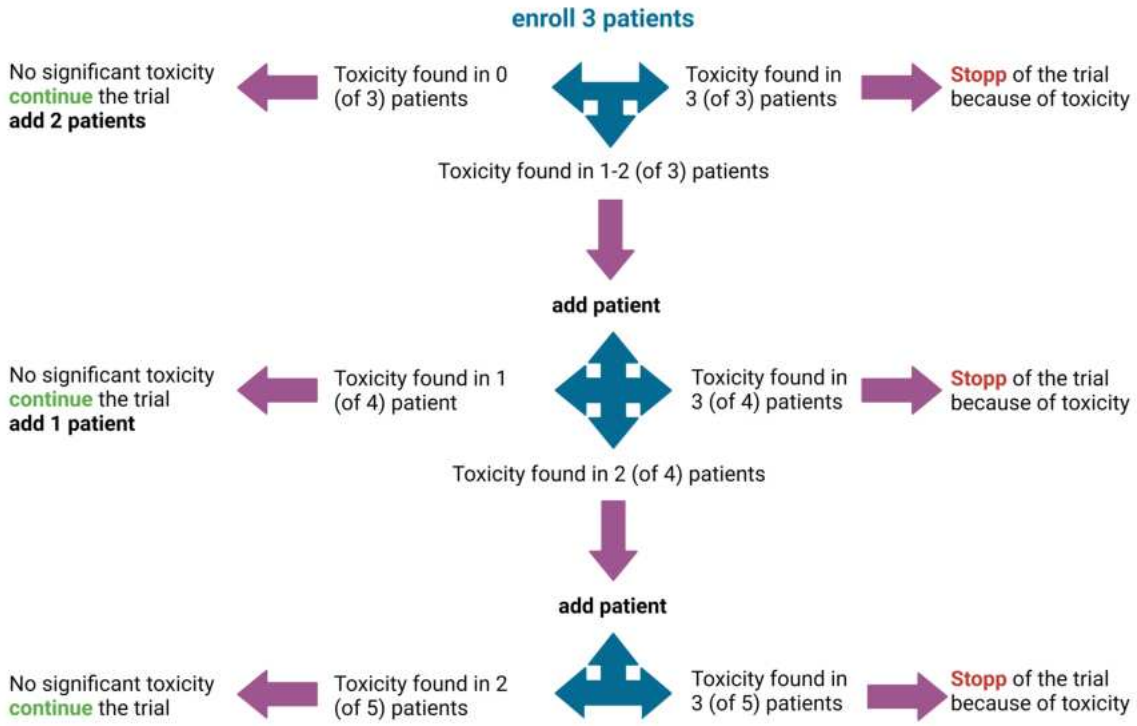

Figure 2: Description of 3+1+1 design for the pilot trial [13]. Created with BioRender.com.

LDRT)
ALS-FRS-R, CSF (Neurofilament light, neuroinflammation kit, 8-OHdG), 3T MRI (QSM), Urine (8-OHdG and p75ECD)
1st post-treatment examination (3 days after completion of treatment with LDRT)
Urine (8-OHdG and p75ECD)

Regular follow-ups according to the StrSchG

Contact every 6 months for the next five years, also by telephone, for questioning about late radiogenic side effects. Possible toxicities will be documented accordingly.

Number of patients

In this trial we adopt a 3+1+1 design for a pilot study (best of five). The procedure to determine the safety of trial is illustrated in Figure 2.

In addition, we try to estimate the change in the man ALS-FRS score where we presume a standard deviation of 10 [14] and a width of the confidence intervals of 6.0. Considering a level of confidence of 80%, we find a sample size of five. We expect no dropouts. If dropouts occur, we will enroll missing patients. As Stopp criteria we define side effects of Grade IV according to CTC (Common toxicity criteria).

Treatment

LDRT planning will be performed using a planning CT (dose contribution about 9 mGy) with a slice thickness of 5 mm and in headfirst supine (HFS) position. The patients will be immobilized using the clinical equipment for craniospinal RT (Orfit All-In-One immobilization board by Orfit Industries, Wijnegem, Belgium; thermoplastic head-shoulder mask and reference point at sternum level). For delivery of LDRT, helical tomotherapy will be used. To verify patients' position the surface-tracking system AlignRT (Vision RT, London, UK) and a single MV-CT (only at first radiation appointment, dose exposure 0,013 Gy) will be performed. The treatment including positioning will last about 8 to 10 minutes, depending on size of target volume. In case of device failure or if the treatment time is not manageable due to patients condition LDRT will be delivered using a standard C-arm LINAC and about 2-3 isocenters with a 15-20 cm distance between them in longitudinal direction utilizing 3-4 static fields. Position verification will be done using the surface-guided workflow of the ExacTrac® Dynamic System (Brainlab AG, Munich, Germany). These techniques will lead to an approximate duration of about 3-5 minutes and some spatial comfort for the patient. Nevertheless, dose concept will be 5 x 0.2 Gy/week, total dose 1 Gy to whole neuroaxis, applied through a 6 MV photon beam.

The clinical target volume (CTV) of the brain and spine is contoured using bone and soft tissue windows in all views (axial, sagittal, and coronal) to ensure adequate coverage. The CTV brain covers the entire brain. The CTV spine covers the spinal canal (including the conus medullaris) up to the second lumbar vertebra. A margin of 3 mm to the CTV is used as the planning target volume (PTV).

We suspect that treatment with LDRT can only affect the eyeballs and causes their dryness. Also there is a low risk ($< 5\%$) that treatment with LDRT may cause cataract. From these reasons, eyeballs and lenses will be defined as organ at risks (OARs). Due to the fact that craniospinal LDRT can not cause damage of other organs, no further OARs will be defined.

Key inclusion criteria:

- Male and female patients $\geq$ 18 years of age with first diagnosis of Spinal or Bulbar-Onset of ALS
- Ability to understand the pilot trial and give an informed consent
- Ability to undergo proposed assessment alone or accompanied by a caregiver
- Ability to follow the 5-days RT regiment, alone or accompanied by a caregiver

Key exclusion criteria:

- Inclusion in another interventional clinical trial
- Previous brain radiotherapy
- Oncologic disease (excluding skin cancer) active or in remission from less than 5 years
- Alcohol and/or other drugs dependence during the previous 12 months (DSM-V or ICD-10 criteria)
- Significant psychiatric disease or cognitive deficits

- Persons not able to give consent
- Active or recent (within 3 months) cerebral infection or haemorrhage
- Immunocompromised status
- Female who is pregnant or nursing or who plans to get pregnant during the course of LDRT (to verify this criteria we will enroll women one year after the climacterium or younger women after pregnancy test (HCG in blood sample)

Benefit-risk assessment

As there are no reports on the effects of low-dose radiotherapy for ALS, we cannot tell exactly what benefits this treatment might have for the ALS patients. In patients with Alzheimer's disease, which is also a neurodegenerative disease, treatment with low-dose radiotherapy had positive results with good tolerability. It is possible that after low-dose radiation the disease will progress more slowly for a certain period of time and in some cases there may even be an improvement in existing symptoms. From experience with radiation therapy for other benign diseases, it is to be expected that a possible positive effect will diminish over time. However, it is impossible to say how long this possible effect will last, as there is also a wide individual range in the treatment of other benign diseases. A repetition of low-dose radiation is not planned as part of this clinical trial.

During the study following invasive procedures will be performed in addition to the standard treatment:

Lumbar puncture: the first lumbar puncture is performed as part of the treatment according to current guidelines; the second lumbar puncture at week 10 is performed as part of this clinical trial. The risk associated with the additional lumbar puncture is identical to the risk associated with the first one as part of the standard treatment.
Most common side effects (according to the information sheet):

- Occasionally injury to small blood vessels
- Occasionally brief ischialgia-like pain due to irritation of a nerve root by the puncture needle
- Loss of consciousness
- Cerebrospinal fluid hypotension syndrome: headaches, sensitivity to light, nausea, vomiting and back pain
- Temporary hearing loss, tinnitus, ear pressure

MRI with contrast agent: The first MRI of the skull with contrast agent is performed as part of the treatment according to the current guidelines; the second MRI with contrast agent at week 10 is performed as part of this clinical study. The risk associated with the additional examination is identical to the risk associated with the first examination as part of standard treatment.
Most common side effects (according to the information sheet):

- First occurrence of claustrophobia (fear of confined spaces) or panic attacks
- Bruising or post-operative bleeding after administration of contrast agent
- Infections at the injection site
- Allergy

In very rare cases, a serious, untreatable connective tissue disease can develop after administration of contrast agent.

LDRT: Low-dose irradiation of the entire neuroaxis (brain + spinal cord) is carried out as part of this clinical trial. The mental impairment caused by low-dose radiation is based on observational data from uncontrolled radiation exposure and cannot be compared with strictly controlled low-dose radiation therapy. We expect slight fatigue as part of the therapy, but not other side effects. Theoretically, there is an increased risk of tumor development with every radiation treatment. These tumors usually only develop after a longer period of time (approx. 10 years, 1:1000). This applies in particular to children who have been irradiated with higher doses.

Blood sampling: Blood sampling is a routine test and is carried out in accordance with guidelines as part of preventive care and general medical treatment. There is no increased risk due to the additional blood sampling during this clinical trial. For pre-menopausal women (including 1 year after the last menstrual period), an additional 8 ml tube will be taken for a pregnancy test.
Most common side effects of blood sampling:

- Formation of a hematoma
- Swelling
- Pain
- Allergic reactions to the materials used (very rare)

Since the expected side effects are considered to be very rare, but the expected benefits could significantly improve the quality of life, we consider this concept to be medically justifiable.

Termination criteria for the trial are adverse events according to the Common Terminology Criteria for Adverse Events (CTCAE) Grade IV related to the study treatment as defined by an experienced physician. Such adverse events are classified as life-threatening and usually require immediate medical intervention. In such patients the treatment will be interrupted and the patients are referred for further treatment, if needed. Patients can terminate the study at any time. In such cases these patients will receive follow-up visits according to the study protocol.

Patient Confidentiality and Data Protection

Patient identifiable data, including initials, date of birth, sex and postcode will be collected by Department of Radiation Oncology, University Hospital Magdeburg. This institution will preserve patient confidentiality and will not disclose or reproduce any information by which patients could be directly identified. Data will be stored in a secure manner in accordance with The Euopean Union The General Data Protection Regulation (GDPR) law. Data will be stored on internal password logged devices which can only be accessed by the accredited persons. Blood sample sent for further analyses to third party institutions will be pseudomized while the respective key remains at the university hospital Magdeburg. The key will be deleted at the end of trial. Patient data will be stored for a maximum of 15 years and can be deleted upon patient request. Patient data will not be transferred to other parties.

Pilot Trial Road Map

1st week

Department of Neurology

- Information about pilot trial
- Handing out Patient information (s. Supplementary)
- Referral to Department of Radiotherapy

Department of Radiotherapy

- Verification of suitability
- Informed consent for the pilot trial (s. Supplementary)

2nd week

Department of Radiotherapy

- Physical examination (s. Supplementary)
- Medical history survey (s. Supplementary)
- Blood and urine sample (s. Supplementary)

3rd week

Department of Neurology

- Neurological examination
- Baseline diagnostics
  - Lumbar puncture
    - Neuroinflammation ELISA Kit
    - 8-OHdG ELISA Kit
    - Neurofilaments Kit
  - ALS-FRS-R
- Blood and urine sample (s. Supplementary)

Department of Neuroradiology

- 3T MRI

4th week

Department of Radiotherapy

- Planning-CT neurospinal axis prone position
- Planning of radiotherapy of neurospinal axis by physicians and physicists

5th week

Department of Radiotherapy
1st day

- LDRT of neurospinal axis: 1st fraction à 0,2 Gy

2nd day

- Medical examination of early side effects according to CTC (s. Supplementary)
- LDRT of neurospinal axis: 2nd fraction à 0,2 Gy

3rd day

- Medical examination of early side effects according to CTC (s. Supplementary)
- LDRT of neurospinal axis: 2nd fraction à 0,2 Gy

4th day

- Medical examination of early side effects according to CTC (s. Supplementary)
- LDRT of neurospinal axis: 2nd fraction à 0,2 Gy

5th day

- Medical examination of early side effects according to CTC (s. Supplementary)
- LDRT of neurospinal axis: 2nd fraction à 0,2 Gy

6th week (1st day)

Department of Radiotherapy

- Physical examination
- Urine sample

7th-9th week

No interventions

10th week

Department of Neurology

- Follow-up neurological examination
- Post-treatment diagnostics
  - Lumbar puncture
    - Neuroinflammation ELISA Kit
    - 8-OHdG ELISA Kit
    - Neurofilaments Kit
  - ALS-FRS-R
- Blood and urine sample (s. Supplementary)

Department of Radiotherapy

- Physical examination (s. Supplementary)
- Medical history survey (s. Supplementary)
- Examination of late side effects according to CTC (s. Supplementary)

Department of Neuroradiology

- 3T MRI

Every six months for the following five years

Department of Radiotherapy

- Regularly follow-ups regarding late radiogenic side effects (according to RTOG/EORTC criteria)

Unknown

Department of Neurology

- Documentation of the last medical contact or (if known) death of the patient

Informed consent for the clinical trial

Patients with ALS (after neurological evaluation and diagnosis) will be informed about the possibility to participate in this pilot study in Department of Neurology. In case of interest, they will be referred to Department of Radiotherapy, where a radiotherapist will examine them, inform them about the project and obtain informed consent if the patient is willing to participate.

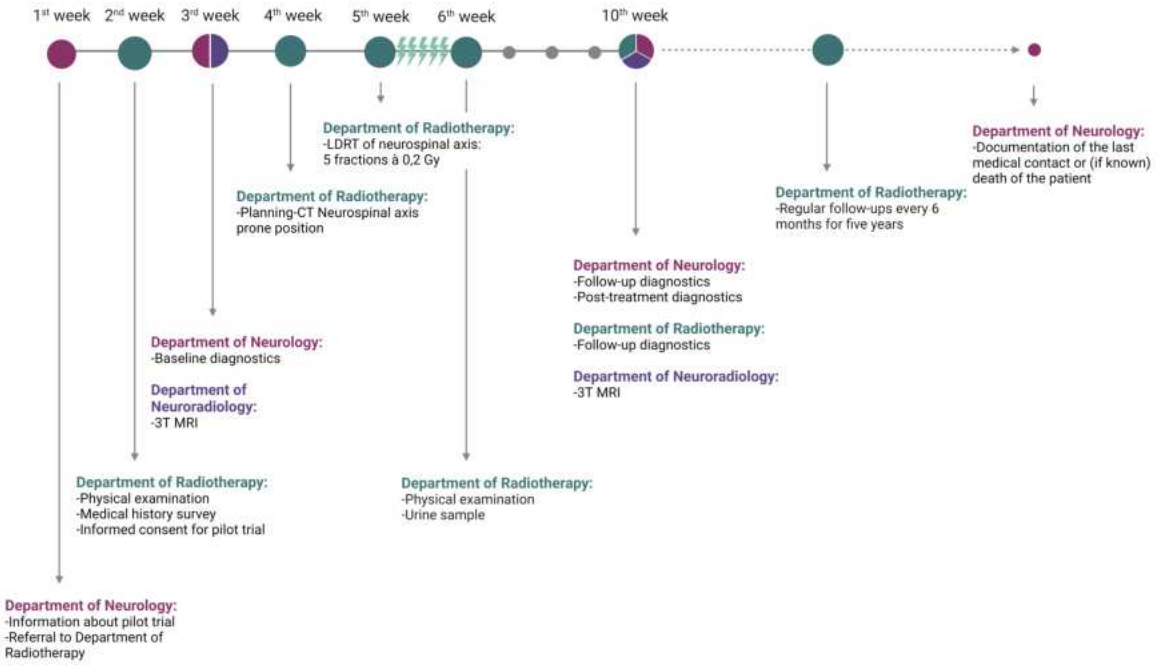

Figure 3: Pilot Trial Road Map. Created with BioRender.com.

Statistics

As this is a feasibility study, no formal statistical analyses will be performed. Key parameters used for future analyses such as mean change in key scores including standard deviation will be estimated. The study design has been outlined above.

Data protection and data flow

The personal and medical data collected as part of this pilot study will be stored for ten years and analyzed in accordance with the currently valid data protection regulations (EU General Data Protection Regulation, Saxony-Anhalt state data protection). The participants will be informed about this in detail in the patient information (during informed consent or in the information provided for the patients). Data access is restricted to the principle investigators (Milanovic, Vasilevska, Medenwald, Schreiber, Vielhaber). The results of this study should be published anonymously. In the case of external shipment, the taken samples would be pseudonymized.

Insurance

During this study patients will be covered by the public liability insurance of the University Hospital Magdeburg A.ö.R. if the damage was caused by the University Hospital. Separate insurance will be taken out for the use of LDRT. Respective documents are attached to this protocol.

Use, transport and storage of biomaterials

Urine and blood from all participants in this study will be stored at -72 °C and analysed in the Department of Neurology. The cerebrospinal fluid obtained from the study participants will also be stored at -72C in the Department of Neurology. Afterwards it will be sent to Düsseldorf University Hospital for further analysis and it will be transported by courier service. After use, all biological material obtained from participants of this trial will be desetroyed.

Storage of MRI

The reports and images from the MR examination will only be available to the doctors involved in this project. According to the current low, all images will be destroyed after 25 years.

Publication Policy

The results of this trial will be presented at relevant conferences and published in a peer reviewed journal. The primary publication from this study will be written by the Chief Investigator and other co-investigators that make a significant contribution to this study. The Clinicaltrials.gov number of the trial and the funder reference number will be quoted in all publications. Data generated from this trial will be the property of Department for Radiotherapy, University Hospital Magdeburg.

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