RESEARCH PROTOCOL

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Neurocognition after radiotherapy in adult brain and base of skull tumours

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1. Study Synopsis

Title of clinical trial	Neurocognition after radiotherapy (RT) in adult brain and base of skull tumours
Protocol Short Title/Acronym	Neurocognition After Radiotherapy in CNS and base of Skull tumours/ NARCiS
Sponsor name	UZ Leuven
Principal Investigator	Maarten Lambrecht, MD PhD
Medical condition or disease under investigation	Neurocognitive decline after RT in adult primary brain tumour patients
Purpose of clinical trial	Develop NTCP model to predict neurocognitive function after radiotherapy in brain and base of skull tumours
Primary objective	Develop an NTCP model for neurocognition after RT
Secondary objective (s)	- Estimate prevalence and severity of neurocognitive decline for all cognitive domains - Identify brain structures or functional brain areas important in neurocognitive decline (based on dedicated MRI). - Search for dose-dependencies of specific neurocognitive skills after RT in adult brain tumour patients - Evaluate correlations between RT dosimetry and early brain changes (MRI)
Trial Design	Multicenter observational cohort study

Participating centers	- UZ Gent - Iridium Kankernetwerk Antwerpen
Endpoints	**Primary endpoints:** - Measure the prevalence and severity of neurocognitive decline compared to baseline at 1 year post-baseline, for all cognitive domains - Predict neurocognitive dysfunction based on RT dosimetric and other explanatory variables **Secondary endpoints:** - Identify early and late changes on structural and functional MR images - Evaluate associations with neurocognitive functioning and RT dosimetry
Sample Size	n=120
Summary of eligibility criteria	Adults (≥18 years) with a primary brain or base of skull tumour, who are amenable for conventionally fractionated radiotherapy (photon or proton irradiation)
Maximum duration of treatment of a Subject	Follow-up for at least 2 years after radiotherapy
Version and date of final protocol	Version 2, 15-06-2022
Version and date of protocol amendments	/

2. Background and rationale

The worldwide incidence rate of primary malignant brain and other central nervous system (CNS) tumours in 2018 was 3.5 per 100,000, representing an overall total of 296,851 individuals[1]. Due to improved treatment strategies, long-term survival can be achieved in an increasing number of CNS patients. Limiting side-effects and safeguarding quality of life after treatment is therefore of utmost importance. Treatment modality of primary brain tumours depends both on the histologic, molecular and genetic characteristics of the tumour but mostly consists of multimodal therapy including surgery, radiotherapy (RT) and/or chemotherapy. One of the most elusive long-term toxicities related to RT of the brain is neurocognitive decline[2]. Deterioration in neurocognitive functioning, including memory loss, concentration disorders, decline in executive, visuospatial and language functioning, places a heavy burden on both the medical and socio-economic aspects of the patients' lives[3]. We observe such a decline in up to half of all these patients treated with RT[4]. Predicting which patients will experience more or less cognitive sequelae is still a challenge.

Preclinical studies have shown that radiation-induced brain injury is due to microstructural modifications at both hippocampal- and non-hippocampal-dependent brain regions[5]. While the exact pathophysiology is complex and poorly understood, the dose delivered to specific (sub)structures is known to correlate with neurocognitive decline[5-7]. For instance, hippocampal dosimetry was associated with memory decline[8], while higher RT dose to frontal areas was associated with a decline in executive functioning such as verbal fluency[9]. However, these findings have not been validated prospectively yet. Despite such increased risk, RT-induced neurocognitive decline and its impact on adult survivors is poorly studied and many knowledge gaps have to be filled. Previous research on long-term neurocognitive side effects associated with chemo- and radiotherapy in CNS cancer patients showed inconclusive outcomes[10-12]. This is partly due to the lack of a standardized neurocognitive follow-up. Even though international guidelines suggested a standard battery for non-CNS cancer patients[13], study designs remain inconsistent, and the sensitivity and specificity of this battery in CNS patients remain inconclusive. Moreover, empirical studies on treating or preventing cognitive impairment in patients with brain tumours have failed to show significant results[14-15].

Recently developed imaging techniques provide not only an opportunity to estimate and visualise RT-induced damage, but they also offer the opportunity to identify new targets for interventions to prevent or lessen cognitive side-effects. Previous neuroimaging studies only focused on micro-, and macrostructural anatomical changes after RT, including grey[16] and white matter[17] (WM) changes. More recent advanced magnetic resonance imaging (MRI) acquisition and analysis techniques, including diffusion-weighted and functional MRI, are nowadays available. They allow us to probe the underlying structural and functional brain network (i.e. "connectome"), respectively. These techniques might be more sensitive to detect possible therapy-induced structural and functional changes in the brain topology, which could relate to cognitive deficits. Furthermore, cognitive impairment may be driven by damage to specific fibre tracts associated with cognitive assessments, which can be modelled based on diffusion-weighted MRI tractography[18].

The proposed observational cohort study will combine the above-mentioned MR imaging techniques together with elaborate neuropsychological assessments and RT dosimetry in 120

patients who will be examined baseline (before RT) and followed longitudinally after RT. By doing so, we hope to uncover vulnerable brain regions and detect early imaging changes to identify patients at risk. Since neurotoxicity is a complex multifactorial process, the best way to combine these data and make predictions about RT-induced effects is to build so-called normal tissue complication probability (NTCP) models. These mathematical models allow us to estimate the risk of a particular side effect in a structured and validated way for every individual patient. This will enable us to simulate the patient's toxicity risks to spare neurocognitive function in daily life. A previous effect has been conducted by Gondi in 2012, but included a limited number of patients and this model has not been validated to date[19].

In this exploratory prospective study, we thus aim to provide the clinical, radiological and dosimetric data to build a validated NTCP model for neurocognitive decline, which is currently lacking.

3. Trial objectives and Design

3.1 Trial objectives

The aim of this trial is to study the long-term impact of multimodal treatment (chemotherapy, radiotherapy and surgery) in adult brain and base of skull tumours on neurocognitive functioning using neuropsychological testing combined with advanced MR imaging techniques in a multicenter observational cohort study. We differentiate two main objectives.

The first objective is to build an NTCP model for neurocognitive decline after RT (for each cognitive domain separately), linking dose-volume parameters to structures within the brain susceptible to neurological damage and neurocognitive decline after radiotherapy. These NTCP models will be used to make predictions on neurocognitive decline in future primary brain tumour patients receiving cranial RT. It will provide us with a much needed, evidence-based tool to select the optimal treatment modality for each individual patient and enables us to tailor cranial irradiation towards neurocognition-sparing treatment in the future.

The second objective is to evaluate dose-dependent neurocognitive decline. In particular, we will investigate:

- Prevalence and severity of neurocognitive decline after RT for all cognitive domains
- Brain structures or functional brain connections important in neurocognitive functioning (based on dedicated MRI).
- Dose-dependencies of specific neurocognitive skills after RT in adult brain tumour patients
- Correlations between RT dosimetry and early brain changes (MRI)

With this prospective observational trial, we aim to map the neurocognitive side effects of the irradiation treatment in cranially irradiated patients and the clinical and dosimetrical factors that play a role. Based on these findings, we plan to set up future prospective trials to validate these findings.

3.2 Primary endpoints

- Predict neurocognitive decline based on RT dosimetric and other explanatory variables like gender, age at diagnosis, comorbidities, level of education, social factors such as social activity and occupation, tumour size and localization, pathological/genetic/molecular characteristics, therapy protocols (surgery, radiotherapy and/or chemotherapy) in an NTCP model for each cognitive domain
- Measure the prevalence and severity of neurocognitive decline compared to baseline at one year, for all cognitive domains (attention, memory, working memory, information/visuomotor processing speed, executive functioning)

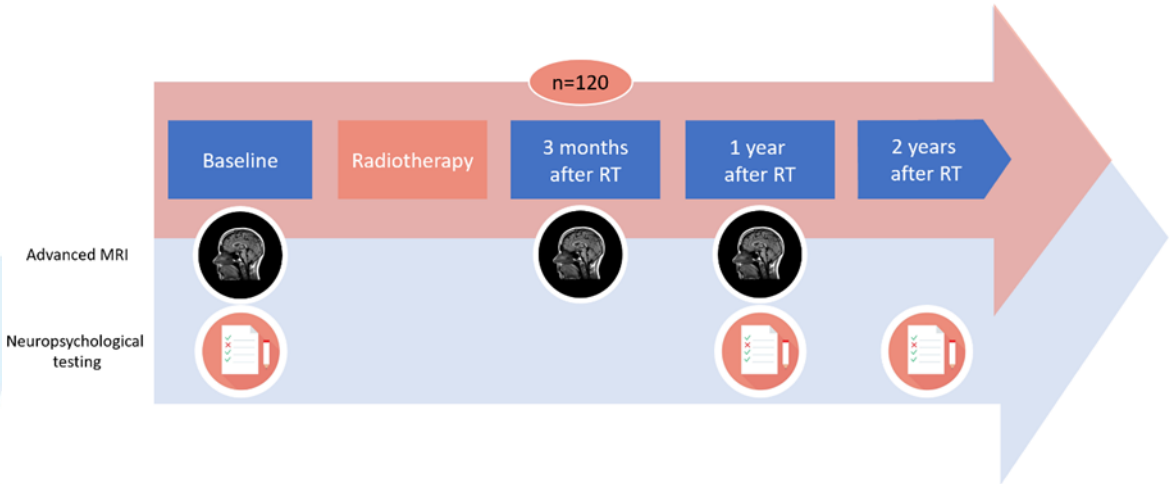
3.3 Secondary endpoints

- Identify early and late changes on structural and functional MR imaging
- Evaluate associations between neurocognitive functioning and RT dosimetry

3.4 Trial Design

This is an observational cohort study which evaluates primary brain tumour patients longitudinally at the following timepoints: baseline (minimal 4 weeks after surgery (if indicated), before radiotherapy), three months (+/- 2 weeks) after end of radiotherapy, 1 year after end of radiotherapy (+/- 1 month) and 2 years after end of radiotherapy (+/- 1 month). At each visit, neurocognitive testing, a self-report inventory and/or advanced MR imaging will take place. During and after radiotherapy and at each follow-up visit, adverse events will be monitored using CTCAEv5.0[20].

3.5 Study Flowchart



4. Selection and withdrawal of subjects

4.1 Inclusion criteria

- Adult patients (≥ 18 years at the time of diagnosis) with a primary brain or base of skull tumour, who are amenable for conventionally fractionated radiotherapy (photon or proton irradiation)

4.2 Exclusion criteria

- Patients with tumours with poor prognostic characteristics
    - Grade IV glioblastoma
    - IDH1/2 wild type glioma
    - grade III meningioma
    - H3K27M midline glioma
- Patients with tumours requiring craniospinal irradiation (CSI)/whole ventricular irradiation (WVI):
    - Medulloblastoma
    - Germinoma
    - Ependymoma requiring CSI
- Hypofractionated/stereotactic radiation (fraction sizes > 2 Gy per fraction)
    - AVM
    - Acoustic schwannoma
    - Brain metastasis
- Inability to perform the cognitive tests or self-report inventories because of motor/sensory deficits or insufficient Dutch language proficiency
- Mental retardation documented before diagnosis
- Pre-diagnosis/pre-existing psychiatric diagnosis resulting in cognitive deficits like psychoses, neurodevelopmental disorders (autism/learning disorders)
- Relapse priory treated by chemo and/or radiation therapy
- Genetic syndrome (e.g. Down)
- Unable to perform MR imaging (claustrophobia, metallic implants like pacemaker/ICD/neurostimulator)

4.3 Expected duration of trial

Each participant will be followed at least for two years after radiotherapy. The trial will be regarded as completed when 120 patients will have reached a follow-up of two years. Since neurocognitive decline is considered to be progressive, participants will still be in follow-up after completion of the trial according to the clinical practice guidelines.

4.4 Subject discontinuation or withdrawal

Participation can be discontinued at any time if the subject, the Investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Screening failure
- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Lost to follow-up
- Sponsor request for early termination of study
- Subject death
- Progressive disease or relapse during follow-up

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. Reasonable attempts will be made by the Investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents and the Case Report Form (CRF). The Investigator will make every effort to contact subjects who are lost to follow-up. Attempts to contact such subjects must be documented in the subject's records (e.g., times and dates of attempted telephone contact, receipt for sending a registered letter, etc.). Subjects who prematurely discontinue are not to be replaced. For subjects considered lost to follow-up, the CRF must be completed up to the last visit performed.

5. Trial Procedures

Subjects will be screened at the consultation of the neurosurgeon (after biopsy or surgery) or after physician referral to the local radiotherapy-oncology centre. This study will be listed at www.clinicaltrials.gov.

5.1 By visit

Each patient will have 5 trial visits. The sequence of procedures to be performed at each visit is detailed below.

		SCREENINGS PHASE	FOLLOW-UP PHASE							END OF STUDY
Visit name	Protocol	Screenings visit	Baseline visit*	RT start	End of RT		3 months after	1 year after RT	2 years after RT	End of study visit°
Day				D0	Dstop		3 M	12 M	24M	24 M
Visit window	3.4	D-28 to 0	D -21 to 0		+/- 3D		+/- 2 W	+/- 4 W	+/- 4W	+/- 8W
Informed consent	IC	x								
Eligibility criteria assessment	4	x								
Patient information and history[a]	R	x								
Tumour characteristics[a]	R	x								
RT charachteristics[a]	R				x					
Questionnaires[b]	5.3		x					x	x	
Neurocognitive testing[c]	5.2		x					x	x	
MRI[d]	5.4		x				x	x		
RT plan[d]					x					
CRF toxicity[a]	6.2			x	x					x

End of study assessment	R									x

*: minimally 4 weeks after surgery
IC: informed consent form
R: REDCap
○: after 2 years or at progression
a: in REDCap (by CTA or MD)
b: in REDCap (online survey, filled in by patient via REDCap platform)
c: in REDCap (by neuropsychologist)
d: to upload coded DICOM images and RT plan in OncoPlace (by CTA)

5.2 Neurocognitive assessment

We will perform a standardized neurocognitive test battery in all patients at baseline (prior to RT) and each consecutive year (1 year and 2 years post-RT). The acquisition of neurocognitive assessments takes approximately 1 hour. Results will be followed over time and compared to normative data [21,22].

All these tests and questionnaires have been extensively described and used to quantify neurocognitive functioning in adults. Both neurocognitive testing and completion of the questionnaires takes place in the hospital.

We will use a broad evaluation of neurocognitive performance.

- The Montreal Cognitive Assessment (MOCA), is a screening instrument for cognitive deficits in multiple domains[23].
- Trail Making Task (TMT)[24], is an executive task which requires to link characters and digits in the correct sequence.
- Digit span (subtest of the Wechsler Adult Intelligence Scale(WAIS))[25] is a working memory task, which requires to repeat digits in same, reversed and sequencing order.
- The Hopkins Verbal Learning Test- Revised (HVLT-R)[26] is a verbal memory task for which participants need to repeat 12 words (three repetitions of free-recall) to assess verbal learning. Afterwards, they are asked to repeat or recognize the memorized words (Delayed recall and recognition).
- Controlled Word Association Test (COWAT)[27] assesses categoric and phonemic fluency. The participant is required to make verbal associations to a category or different letters of the alphabet by saying all the words which they can think of in this category or beginning with a given letter.
- The Stroop Color and Word Test (SCWT)[28] assesses the ability to inhibit cognitive interference that occurs when the processing of a specific stimulus feature impedes the simultaneous processing of a second stimulus attribute.
- The digit symbol substitution test of the WAIS (coding)[29] measures processing speed. This test consists of digit-symbol pairs followed by a list of digits. Under each digit the subject

should write down the corresponding symbol as fast as possible. The number of correct symbols within the allowed time is measured.

Cognitive domain	Neurocognitive test	Outcome measurement
Attention	TMT B	time
Memory	HVLT immediate recall	Sum score - learning
	HVLT delayed recall	Sum score
	HVLT recognition	Good recognition-mistakes
	WAIS IV digit span forward	Total number of series
Executive functioning	COWAT	Sum of words
	SCWT	Interference score
Working memory	WAIS IV digit span backwards	Total number of series
Information/visuomotor processing speed	WAIS IV digit span sequencing	Total number of series
	WAIS IV symbol substitution	Sum score
	TMT A	Time

Cognitive tests grouped per cognitive domain and defined outcome measurement. TMT= trail making test, HVLT= Hopkins Verbal Learning Test, COWAT= Controlled Oral Word Association Test, SCWT= Stroop Color Word Test, WAIS= Wechsler Adult Intelligence Scale

5.3 Questionnaires

Daily life functioning will be examined with neuropsychological questionnaires. This self-report inventory will take about 30 minutes. All participants will complete:

- The EORTC brain cancer module (EORTC QLQ-BN20)[30] to assess health-related quality of life and symptoms in these brain cancer patients.
- The Spielberger State-Trait Anxiety Inventory[31] to assess anxiety levels.
- The Beck Depression Inventory-II[32,33] to estimate depressive symptoms.
- The Cognitive Failure Questionnaire (CFQ)[34] to address cognitive problems in daily life.
- The Behavior Rating Inventory of Executive Function (BRIEF-A)[35] to assess specific problems in executive functioning.
- The EORTC QLQ-C30 questionnaire[36] to assess general quality of life.
- The Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) Scale[37] to investigate an individual's level of fatigue during their usual daily activities over the past week.

- The Pittsburgh Sleep Quality Index (PSQI)[38] assesses sleep quality over a one month time interval.

Socio-economic status (educational level of parents and participants) and medical conditions are evaluated at baseline, 1 year and 2 years post-RT with a separate questionnaire. This questionnaire will take maximum 5 minutes.

5.4 MRI imaging

We will perform an advanced MR protocol in every patient. No gadolinium contrast will be administered. Advanced MRI will be performed in all patients prior to RT (baseline), at three months, and at one year post-RT. All subjects will be imaged on the same scanner in each center (UZ Leuven: 3T Philips with a 32-channel phased-array head coil, located in the radiology department at UZ Leuven). Phantom scans will be used to adapt each protocol in every center. Neuroimaging will consist of:

- 3D high-resolution T1-weighted magnetization-prepared rapid acquisition gradient-echo (MPRAGE) anatomical scan for grey/white matter volumetry
- 3D T2-weighted FLAIR to examine leukoencephalopathy
- Advanced diffusion weighted- imaging (DWI). DWI is a technique enabling the visualization and characterization of the WM architecture via the self-diffusion of water molecules - allowing us to study potential therapy-induced changes in the WM microstructure. Damage to WM structures - for example axonal loss or demyelination - may change quantitative DWI parameters such as fractional anisotropy (FA), mean diffusivity (MD) and diffusion kurtosis.
- Resting state functional MRI (rfMRI) to estimate neuronal activity of the brain at rest (examination with eyes closed). rfMRI uses the vascular response nearby electrically active neurons to indirectly visualize brain activity and allows us to determine the functional coherence (or so-called “connectivity”) between the different brain regions at rest. Participants will therefore be asked to close their eyes during the examination, but not to fall asleep.
- Susceptibility-weighted MR imaging (SWI) to detect radiation therapy-induced cerebral microbleeds.
- Arterial Spin labeling (ASL) to measure tissue perfusion.

The duration of the proposed scan protocol is maximum 60 minutes.

6. Assessment of Safety

6.1 Specification of safety parameters

As the oncological treatments (radiotherapy, chemotherapy and/or surgery) and MR imaging are currently being used as standard of care, the study team does not anticipate subjects experiencing any adverse events solely due to being in the study. This is simply a proposal to evaluate and follow subjects undergoing these commonly performed procedures, both of which have been shown to be safe and approved.

Specifically for the MR imaging, the standard safety measures will be respected. Patients will be instructed to remove all metallic objects from pockets and hair, as well as metallic jewellery. Additionally, any individual that goes into the MRI scanner room will be required to follow these same instructions and procedures. Patients will be asked to fill out a screening form asking about anything that might create a health risk or interfere with the MRI exam. Items that may create a health hazard or other problem during an MRI include:

- Certain cardiac pacemakers or implantable cardioverter defibrillators (ICDs)
- Ferromagnetic metallic vascular clips placed to prevent bleeding from intracranial aneurysms or blood vessels
- Some external or implanted medication pumps (such as those used to deliver insulin, pain-relieving drugs, or chemotherapy)
- Certain cochlear (i.e., for hearing) implants
- Certain neurostimulation systems
- Catheters that have metallic components
- A bullet, shrapnel or other type of metallic fragment
- A metallic foreign body located within or near the eye (such an object generally can be seen on an x-ray; metal workers are most likely to have this problem)

In addition, subjects may be harmed by:

• Loud noise from the MRI device. All subjects will be given ear protection to prevent risks from loud noise.
• Some subjects may experience mild transient vertigo and/or metallic taste when they are being moved into the MRI system.

6.2 Procedures for recording and reporting adverse events (AE)

At the beginning and end of radiotherapy and every follow-up visit, a case report form (CRF) will be filled out by the radiation oncologist, scoring symptoms/adverse events using CTCAEv5.0[20] for headache, nausea, epilepsy, gait impairment, dysphasia, dry eye, eye pain, tinnitus, vertigo, vestibular disorder, hearing impaired, vomiting, fatigue, dermatitis, pruritus, scalp pain and cranial nerve impairment.

7. Statistics

7.1 Sample size

Study subjects will be recruited from the radiation-oncology center of three centers: UZ Leuven, UZ Gent and Iridium Kankernetwerk Antwerpen. From a pragmatic perspective, we estimate to recruit 12-15 patients yearly in each of the three centers, resulting in 120 patients in three years in total.

Cognitive impairment has been reported in primary brain tumour patients and is roughly estimated to be present in about half of patients[39-40]. Although limited (good quality) evidence exists for our

type of study, power calculations were performed based on the two most extreme studies (i.e. one showing minimal amount of cognitive impairment[41] and one showing maximal amount of cognitive impairment[42]. In both studies cognitive performance is assessed at baseline and about 12 months after radiotherapy in primary brain tumours. In the study by Moretti et al[42], we used the cognitive outcome results of patients 9-12 months after radiotherapy (45-60 Gy), since the studied population will receive radiotherapy doses within this range. For power calculations, we used the performance on the digit span forward (processing speed) since the prevalence of impairment on this test was low (<20%). Based on these studies, effect sizes of d= 0,368 and d= 1,271 were found with a power of 0,800 and 0,868 respectively. This power would be 0,979 and 1,000 in our specific explorative study, in case of 120 inclusions. In order to detect this difference after 12 months with a power of 0.800 and a two-sided significance level α=0,05, the total required sample size would thus range between 8-60 patients. Power calculations were performed using Gpower 3.1[43].

Sample size calculation for the prediction models was performed using a recently developed software package[44]. Since the event rate of cognitive impairment is unclear, we performed power calculations for the lowest and highest event rate, i.e. 0,2-0,5 (see above). When considering 4 candidate predictors and rMSPE of 0,1, the required sample sizes ranges between 90 and 120 patients respectively. Therefore, including 120 patients will allow building a sufficiently complex prediction model combining four dosimetric and/or clinical features.

7.2 Analysis

Analyses and statistical methods will be applied to all subjects.

7.2.1 Neuropsychological data

The following research questions are addressed by using multiple statistical tests.

1. How severe is the neurocognitive decline after treatment and how does it change over time?
2. Which neurocognitive domains are affected in which patients?

The first question is addressed by investigating the neurocognitive performance of primary brain and base of skull tumour patients. The performance on neurocognitive measures of the patients will be assessed at 3 timepoints over time. The raw test-scores are converted to normalized z-scores based on normative data for each cognitive test. For each cognitive test, cognitive impairment is defined as a z-score ≤1.5, and patients will be dichotomized according to their impairment status (impaired versus unimpaired). The frequency of impairment will be reported as descriptive information.

We will assess the evolution in the z-scores with a mixed repeated measures model to test changes throughout time. However, if the data are not normally distributed, the Friedman test will be used as alternative approach. The effects of the patient- and treatment-related predictors on the neuropsychological and questionnaire scores will be investigated using general linear models (predictors= tumour location, radiation dose, covariates= gender, age at treatment, time since treatment and socio-economic status) at each timepoint. Bonferroni correction will be applied as

method for statistical correction of multiple tests, for each test outcome (n=5 outcomes; memory, executive functioning, attention, visuomotor function, working memory) that is predicted.

Effect sizes of the tests will be reported. Partial eta squared (hp2) will be computed to estimate effect sizes (weak effect: $\eta p^2 \approx 0.03$; moderate: $\eta p^2 \approx 0.06$; large: $\eta p^2 \geq 0.14$) for linear relationships between predictor and each outcome (e.g. age, time since treatment). Cohen's d will be used to estimate effect sizes for subgroup differences (small effect: $0.2 < d < 0.3$; medium: $0.3 < d < 0.8$; large: $d > 0.8$).

7.2.2 Imaging

State of the art image analysis approaches are applied to process and analyze the acquired brain images and to document individual changes in structural white matter or resting state brain activity. Structural MRI data will be analyzed via voxel based morphometry (VBM) in Statistical Parametric Mapping (SPM). Multiple validated toolboxes will be used (e.g. FreeSurfer, ANTs, CAT12). To correct for distortions caused by the brain tumour itself, Virtual Brain Grafting will be used[45]. DWI images will be processed and analyzed using MRtrix 3.0. Seed-based analysis and independent component analysis (ICA) will be applied to analyze the resting state fMRI data. The association between the obtained MR parameters and the performance on neurocognitive tests will be investigated using (voxel- and region of interest-based) correlation analyses. The independent effects of the predictors (radiation dose, gender, age at treatment, duration of treatment and socioeconomic status) on these neuropsychological test scores and imaging feature values will be analyzed with a (voxel-based) general linear model. For the MR analyses, repeated measures ANOVA will be conducted voxel-based to analyze the within-subject variability in tissue probability maps throughout time (e.g. grey matter or white matter volume, grey matter density, white matter microstructure). The significance threshold will be set at $p < 0.05$ false discovery rate (FDR) corrected for multiple comparisons.

7.2.3 Radiation dosimetry

In order to examine the effect of mean equivalent dose in 2Gy fractions (EDQ2) to various brain structures and associations with impairment status, univariate logistic regression analyses will be undertaken. Wald tests will be used to test the statistical significance of each model, using two-tailed tests. P-values <0.05 will be considered significant.

7.2.4 NTCP modeling

Two NTCP modelling efforts will be conducted for each cognitive outcome domain (attention, memory, executive functioning, working memory, Information/visuomotor processing speed). In the first effort, dose metrics of anatomical structures derived from a brain atlas will be used together with clinical patient factors to develop a prediction model of neurocognitive decline. First, univariable logistic regression analysis will be performed to select clinical parameters associated with cognitive outcome. These variables will be ranked based on their association (area under the curve (AUC) of the univariable logistic regression) with the endpoint. The best ranked variable will be selected first. Subsequently, each next variable will be excluded from the ranking if its Pearson's correlation is $r > 0.8$ with any previously selected variable. For the remaining variables, we will check whether non-linear transformations (log, square root, inverse transformations, etc.) improves the association. Considered clinical variables in model building are: gender, age at diagnosis, tumour location, treatment modality, pathology, education, time since treatment. The added value of dosimetry to the use of clinical information will be verified in three steps. Initially,

a multivariable prediction model (logistic regression model with LASSO regularization) will be built with only the clinical variables using a forward stepwise procedure with p = 0.05 (deviance criterion) as critical p-value to stay in the model. Afterwards, the clinical prediction model will be extended by a stepwise addition of dosimetric variables which are ranked in the univariable analysis. In this multivariable prediction model (LASSO), a maximum of 4 variables will be included in each model. Finally, we will test in all models whether proton beam therapy (PBT) itself has an additional independent impact on the outcome risk apart from the already selected dose metrics. If that is the case, this will be investigated further by linear energy transfer (LET) and relative biological effectiveness (RBE) weighted PBT dose maps instead of the standard (RBE=1.1) PBT dose maps. The discriminative ability of the prediction models will be quantified using the AUC and compared between nested models using the likelihood ratio test. To obtain stable prediction models, all modelling steps will be repeated in a 100 times repeated 5-fold cross-validation process. The most frequently obtained models will be selected. Final model coefficients will be determined by fitting these selected models on the complete development dataset. Correction for optimism will be performed using 500 bootstrap samples.

In a second effort, relevant 3D clusters of brain voxels resulting from the brain damage susceptibility heat maps will be included in the modelling. Dose to these brain areas will be used as alternative dosimetric input parameters and the resulting model's performance will be compared to that obtained in the first step. The final NTCP model parameters will be calculated for the whole dataset of patients and per institution in order to detect potential heterogeneity.

Internal validation of the model will be performed using bootstrapping. After external validation, the final NTCP models could possibly be included in decision support software. In case of conflicting neurotoxicity constraints, several 'optimal' solutions to the planning problem (prioritizing one or another brain region) might indeed be possible. This will facilitate plan comparisons and informed decisions on the optimal treatment plan in future patients.

8. Quality assurance

The Principal Investigator will be responsible to ensure the study is conducted in accordance with the protocol, Good Clinical Practice (GCP), applicable regulatory requirements, and that the data recorded is valid. To achieve this objective, the study will be continuously monitored and reviewed monthly by the study team.

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial follows the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

MR quality will be checked with initial phantom scans acquired at each site. The neuro-MR protocol will also be tested and optimized at each participating center.

9. Direct access to source data and documents

The investigators and the institutions will permit trial-related monitoring, audits, EC review, and regulatory inspections (where appropriate) by providing direct access to source data and other documents (ie patients' case sheets, MR reports, histology reports etc).

10. Ethics and regulatory approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (current version), the principles of GCP and in accordance with all applicable regulatory requirements. This protocol and related documents will be submitted for review to Ethics Committee for Clinical Trial Authorisation.

The Study can and will be conducted only on the basis of prior informed consent by the Subjects, or their legal representatives, to participate in the Study. The Participating Site shall obtain a signed informed consent form (ICF) for all patients prior to their enrollment and participation in the Study in compliance with all applicable laws, regulations and the approval of the Ethics Committees (UZ Leuven, UZ Gent and GZA). The Participating Site shall retain such ICFs in accordance with the requirements of all applicable regulatory agencies and laws.

The Investigator and the Participating Site shall treat all information and data relating to the Study disclosed to Participating Site and/or Investigator in this Study as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the performance of the Study. The collection, processing and disclosure of personal data, such as patient health and medical information is subject to compliance with applicable personal data protection and the processing of personal data (Regulation (EU) 2016/679 also referred as the General Data Protection Regulation ("GDPR") and the Belgian Law of July 30 2018 on the protection of natural persons with regard to the processing of personal data).

11. Data Handling and Management

The collection of personal patient information will be limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected. Only study personnel will collect data. Klinisch Werk Station (UZ Leuven) and EPD (GZA, UZ Gent) will be used to assemble the clinical data of the patients who are eligible and willing to participate. The dosimetric data (e.g. mean EDQ2 of delineated structures) will be extracted from the therapy planning system (ARIA and Raystation).

An excel file containing the data of all eligible patients will only be saved on the internal server of the local hospital (password protected). We will collect the following personal information: EAD number (patient verification number of KWS), date of birth, pathology and date of diagnosis.
This dataset will be coded (pseudonymized) in a separate password-protected excel file. These coding documents, which associate patient ID numbers with their individual code will only be saved on the internal server of the local hospital (GZA, UZ Gent and UZ Leuven).

The pseudonymized data per patient (without patient identifiers) will be complemented with additional clinical information, stored and shared by the study team using the REDCap data

management system. The clinical data which will be collected, are: gender, age at diagnosis, comorbidities, BMI, medication and alcohol/nicotine (ab)use, level of education, social factors such as social activity and occupation, tumour size and localization, pathological/genetic/molecular characteristics, therapy protocols (surgery, radiotherapy and/or chemotherapy) and radiotherapy doses. The researchers of KU and UZ Leuven will have access to the data of all patients (for analysis purposes). The researchers of UZ Gent and Iridium Kankernetwerk Antwerpen will have limited access to the data (only data of the patients they recruit themselves).

Hard copy documents will be retained for the duration of the study until data entry. All hard copy documents (informed consent, neurocognitive test forms, surveys) will be kept in a locked cabinet in the research coordinator's office.

Radiotherapy treatment plans and MR-images of the Sponsor and Participating Sites will be stored and shared via a secured web-based platform dedicated to the review of images & RT treatment reviews and the management of clinical trials, AQUILAB OncoPlace. Pseudonymisation of these data can be acquired on different levels: data can be exported without patient identifiers from the MR scanner. Another option is to use the softwarepackage MRIcroGL which deletes the patient identifiers from the acquired images.

Analyses will be conducted on the pseudonymized dataset only. To perform statistical tests, the Statistical Package for the Social Sciences (SPSS) is used.

The database is managed and supervised by the Principal Investigator. Coded data are only possible to be shared with involved researchers from UZ Leuven after the pseudonymization stage. All hard copy documents will be shredded within 25 years after completion of the study upon Sponsor approval.

12. Publication Policy

It is anticipated that the results of the overall Study shall be published in a multi-centre publication, involving the data of all clinical sites participating in the Study.

Participating Site is not allowed to publish any data or results from the Study prior to the multicentre publication, provided however that Participating Site is allowed to publish the results generated at the Participating Site if the multicentre publication has not occurred after 12 months from Study database lock.

Any publication by Participating Site will be submitted to the Sponsor for review at least thirty days prior to submission or disclosure. Sponsor shall have the right to delay the projected publication for a period of up to three months from the date of first submission to the Sponsor in order to enable the Sponsor to take steps to protect its intellectual property rights and know-how.

Publications will be coordinated by the Investigator of Sponsor. Authorship to publications will be determined in accordance with the requirements published by the International Committee of Medical Journal Editors and in accordance with the requirements of the respective medical journal.

13. Insurance/Indemnity

In accordance with the Belgian Law relating to experiments on human persons dated May 7, 2004, Sponsor shall assume, even without fault, the responsibility of any damages incurred by a Study Patient and linked directly or indirectly to the participation to the Study, and shall provide compensation therefore through its insurance.

14. Financial Aspects

This research project is funded by Kom op tegen Kanker (Stand up to Cancer), the Flemish cancer society.

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