Study Protocol

Health Effects of C**A**rdiac Fluo**R**oscopy and **MO**der**N** Rad**I**otherapy in Pediatri**C**s (**HARMONIC**) – **Radiotherapy (WP2 / WP4 / WP5)**

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AUTHORS OF THIS DOCUMENT

Name	Institute	Country
Neige JOURNY	Inserm	France
Julie NYS	Inserm	France
Siamak HAGHDOOST	Stockholm University	Sweden
Lorenzo BRUALLA	Westdeutsches Protonentherapiezentrum Essen	Germany
Yasmin LASSEN	Aarhus University Hospital	Denmark
Louise TRAM HENRIKSEN	Aarhus University Hospital	Denmark
Isabelle THIERRY-CHEF	ISGlobal	Spain
Beate TIMMERMANN	University Hospital Essen; Westdeutsches Protonentherapiezentrum Essen	Germany
Nadia HADDY	Gustave Roussy	France
Maria Grazia ANDREASSI	Institute of Clinical Physiology	Italy
Brice FRESNEAU	Gustave Roussy	France

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OVERVIEW OF THE HARMONIC CONSORTIUM

Objectives of the HARMONIC Consortium

The use of radiation for medical diagnosis and treatment procedures has had a major impact on the survival of paediatric patients. Although the benefits of these techniques largely outweigh the risks, there is a crucial need to better understand the long-term health effects of such exposures in order to optimise treatment plans in these young patients and to reduce the risk of late toxicities.

HARMONIC, a consortium of 24 European partners, is a European-funded project built up to better understand the long-term health effects of medical ionizing radiation exposure in children and adolescents, focusing on two different and complementary populations: (1) Paediatric patients undergoing modern radiotherapy (including proton therapy); (2) Paediatric patients undergoing interventional cardiology. By building European cohorts and registries for long-term follow-up of paediatric patients, HARMONIC aims to:

- Investigate the long-term health effects of ionising radiation exposures in children and adolescents;
- Provide the medical and radiation protection communities with tools for long-term follow-up of children and adolescents exposed to medical radiation ;
- Improve estimates of radiation doses to specific organs ;
- Investigate possible biological mechanisms leading to the development of health effects in these patients later in life ;
- Establish recommendations to optimise radiotherapy and interventional cardioliogy treatments in paediatric patients, and further reduce radiation doses.

HARMONIC will provide much needed information on the effects of low to high doses of radiation exposures in children and adolescents. This knowledge will be important to improve radiation protection in medicine. The project is based on building a close relationship between clinicians, radiation protection scientists, sociologists and patients, which will ensure the study is relevant not only in terms of clinical effectiveness but also in terms of patient care and quality of life.

List of Work Packages

HARMONIC encompasses six distinct and complementary work packages (WPs – see Figure 1), including dosimetry (WP4) and biology (WP5), which are fully integrated in the project, with activities contributing to answering the main questions addressed in the two epidemiology work packages (WP2, WP3).

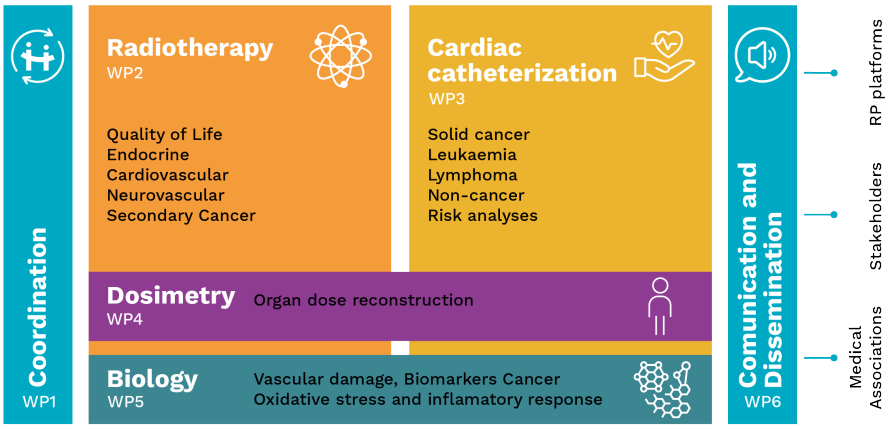

Figure 1: Organization of the HARMONIC Consortium

SCOPE OF THE PROTOCOL

HARMONIC is a collaborative project, which aims at better understanding the long-term health and social effects of medical exposure to ionising radiation in children and adolescents, specifically cancer patients treated with modern radiotherapy techniques and cardiac patients treated with X-ray guided imaging procedures.

HARMONIC is coordinated by ISGlobal, Barcelona, Spain (Dr. Isabelle Thierry-chef).

HARMONIC is structured into 6 WPs: WP1: Coordination, **WP2: Radiotherapy**; WP3: Interventional Cardiology, **WP4**: Dosimetry (for **Radiotherapy** and Interventional Cardiology), and **WP5**: Biology (for **Radiotherapy** and Interventional Cardiology), and WP6: Communication and Dissemination.

This present document only refers to research activities in WP2, WP4 and WP5 on late effects of radiotherapy in patients with cancer. WP1 and WP6 refer to project management and communication activities. WP3 aims at building a European cohort of children, adolescents and young adults treated with interventional X-ray procedures used for cardiac diseases. WP1, WP3 and WP6 are out of the scope of the present document.

WP2 aims at building a registry of paediatric patients treated with modern radiotherapy techniques in Europe, and investigating late health effects of low, moderate and high radiation doses from modern external radiotherapy techniques using photons or protons in paediatric patients.

WP4 aims at estimating individual, in-field and out-of-field radiation doses delivered to the whole-body and specific organs of interest during radiotherapy.

WP5 aims at investigating mechanisms and at identifying potential biomarkers that can be used a) for molecular epidemiology to refine risk estimates for adverse health effects/disorders b) for individualised therapy or providing a rationale for selection of optimal diagnostic/therapeutic methods. Focus will be on oncogenic processes and vascular diseases.

WP2, WP4 and WP5 are inter-connected. WP2 provides the infrastructure and data (radiotherapy data and biological samples) needed for the development of WP4 and WP5's activities on patients treated with radiotherapy, and benefits from the outputs of these activities (radiation dose estimates, and blood/saliva biomarkers) to conduct its own research activities. WP5 also uses the radiation dose estimates delivered by WP4-task 4.2. Consequently, the present document presents all activities that are developed in WP2, and the WP4 and WP5's Radiotherapy part, including their interconnections in terms of data processing and protection.

HARMONIC WP2 is co-led by Prof. Dr. med. Beate Timmermann (University Hospital Essen, Germany), and Dr. Neige Journy (Inserm, France). WP4-task 4.2 Radiotherapy is led by Dr. Lorenzo Brualla (WPE, Germany), and WP5 is led by Dr. Siamak Haghdoost (Stockholm University, Sweden).

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LIST OF ABBREVIATIONS

3DCRT	Three-dimensional Conformal Radiotherapy
8-oxo-dG	8-hydroxy-2′-deoxyguanosine
BNP	B-type Natriuretic Peptide
CépiDC	French registry of causes of death
CNS	Central Nervous System
CPK	Creatine PhosphoKinase
CRF	Case Report Form
CSI	Craniospinal irradiation
CT	Computerized Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DICOM	Digital Imaging and Communications in Medicine
DICOM-RT	Digital Imaging and Communications in Medicine - Radiation Therapy
EBRT	External Beam Radiation Therapy
EORTC	European Organisation for Research and Treatment of Cancer
EPTN	European Particle Therapy Network
ESTRO	European SocieTy for Radiotherapy and Oncology
EU	European Union
EURADOS	The European Radiation Dosimetry Group
EURAMED	European Alliance for Medical Radiation Protection Research
GnuPG	Gnu Privacy Guard
HARMONIC	Health Effects of Cardiac FluoRoscopy and Modern RadIotherapy in PediatriCs
HRQoL	Health-Related Quality of Life
ICD	International Classification of Diseases
ICH-GCP	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice
ICMJE	International Committee of Medical Journal Editors
IEC	Independent Ethics Committee
IMPT	Intensity-Modulated Proton Therapy
IMRT	Intensity-Modulated Radiotherapy
INSERM	French Institute of Health and Medical Research.
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
MEDIRAD	Implications of Medical Low Dose Radiation Exposure
miRNA	microRNA
MRI	Magnetic Resonance Imaging
mtDNAcn	mitochondrial DNA copy number
PBT	Proton Beam Therapy
PedsQL™	Pediatric Quality of Life Inventory
PI	Principal Investigator
PROS	Paediatric Radiation Oncology Society
PTCOG	Particle Therapy Co-Operative Group
PTX3	Pentraxin 3
RBE	Relative Biological Effectiveness
RBE	Relative Biological Effectiveness
RNCE	French childhood cancer registry
RNIPP	French population-based mortality registry
ROS	Reactive Oxygen Species
RPPA	Reverse Phase Protein Arrays
SIOP	International Society of Paediatric Oncology
SNIIRAM	French hospital and health insurance database
TNF	Tumor Necrosis Factor

TPS	Treatment Planning Systems
VMAT	Volumetric Modulated Arc Therapy
WP	Work Package

TABLE OF CONTENTS

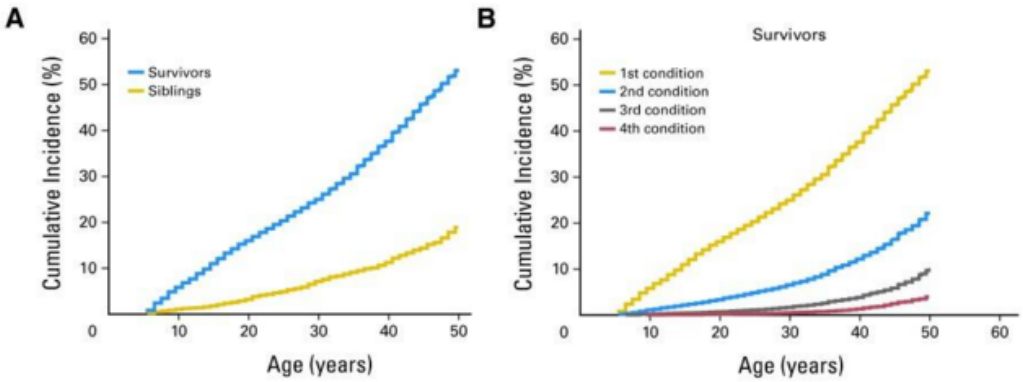
1 SCIENTIFIC RATIONALE

1.1 Childhood and Adolescent Cancer Survivorship: The Burden of Late Morbidities

1.1.1 Overview

Each year, 15.6 / 100 000 children and adolescents (0–19 years of age) are diagnosed with cancer worldwide, of whom more than 60% with leukemia, lymphoma or a tumor of the central nervous system (CNS) [1]. With improvements of cancer detection, treatment and supportive care, survival rates of childhood and adolescent cancers have considerably increased over the past decades [2, 3]. In high-income countries, the current five-year survival rate is about 85% for all age- and diagnosis-specific groups combined, with the vast majority of patients being cured of their original malignancy. Consequently, the number of long-term survivors has considerably increased. It is estimated that 300 000–500 000 individuals living in Europe and 430 000 individuals in the United States, as of 2015[1], are survivors of a cancer diagnosed during childhood or adolescence.

Despite of good survival rates overall, childhood and adolescent cancer survivors are at high risks of developing severe late morbidities due to cancer or treatment sequelae during the years or decades following primary cancer diagnosis. During the first years after diagnosis, cancer recurrence or progression is the leading cause of death. However, rates of mortality from other causes increase over time, exceeding cancer recurrence/progression-related mortality after 30 years of follow-up, and are far beyond that observed in the general population [4]. Half of five-year childhood and adolescent cancer survivors treated in the 1970-80's developped a severe, disabling or fatal health condition by age 50, which is five times higher than among siblings (Figure 2) [5]. A wide spectrum of late adverse outcomes of childhood and adolescent cancers has been reported, the most frequent and severe of them being second primary cancers, cardio- and neurovascular diseases and endocrinopathies [6]. About 20% and 15% of childhood and adolescent cancer survivors treated in the 1970-80's developped, respectively, a second primary cancer or a cardiac disease by age 50 [5]. The 25-year cumulative incidence of stroke after brain tumor was estimated to be about 6% overall [7]. About half of childhood cancer survivors developed an endocrinopathy, with most patients needing lifelong follow-up by an endocrinologist to minimise the effects on growth, pubertal development, bone health, and quality of life [8, 9].



Cumulative incidence of chronic health conditions for (A) grades 3 to 5 chronic health conditions, (B) multiple grade 3 to 5 conditions in survivors, among 14 359 five-year survivors diagnosed with a 1st cancer before age 21 years in 1970-1986 (U.S. Childhood Cancer Survivor Study).

Source: Armstrong GT, Kawashima T, Leisenring W, Stratton K, Stovall M, Hudson MM, Sklar CA, Robison LL, Oeffinger KC. Aging and risk of severe, disabling, life-threatening, and fatal events in the childhood cancer survivor study. J Clin Oncol. 2014 Apr 20;32(12):1218-27.

Figure 2. The burden of long-term morbidities among childhood and adolescent cancer survivors

[1] source: U.S. National Cancer Institute, https://www.cancer.gov/types/childhood-cancers/ccss, last accessed on 12 January 2020

As a consequence, cancer and treatment sequelae had a substantial impact on childhood and adolescent cancer survivors's social life, especially those who were treated for a CNS tumor. Several reports in Europe and the United States showed that these individuals had lower academic attainment, more frequently required special education services, and were more likely to be unemployed and unmarried, compared to their siblings or the general population [10, 11].

The considerable improvement in survival rates observed between the 1970s and the 1990s was primarily attributable to reduced mortality due to recurrence or progression of primary cancer [3]. For some cancer types (e.g. leukemia, Hodgkin's lymphoma, astrocytoma and Wilms tumor), there was also a reduced treatment sequelae-related mortality over time, which was attributable to decreased radiotherapy and chemotherapy exposures, but not for all cancer types. Despite of the use of more advanced radiotherapy techniques and lower chemotherapy doses, no, or very little reduction in treatment sequelae related-mortality was observed for tumors such as medulloblastoma, non-Hodgkin lymphoma, rhabdomyosarcoma, and bone tumors. For certain tumor types, primarily neuroblastoma, there was an even increase in all-cause and treatment sequalae related-mortality in more recent decades, presumably attributable to increased therapeutic intensity that resulted in improved five-year survival. Noticeably, with the exception of Hodgkin lymphoma's survivors, the cumulative incidence of second primary cancers remained unchanged in patients treated in the 1990s compared to those treated earlier [12]. While little improvement in reducing late treatment sequelae-related mortality has been observed in the past decades, expectations of new therapeutic strategies and techniques to reduce late sequelae are high. **Reducing the burden of treatment-related late sequelae in childhood and adolescent cancer survivors, and improving their quality of life and their social integration throughout the lifespan, represents a major challenge of new therapeutic strategies and techniques, including radiotherapy**.

The high burden of long–term morbidities makes long-term documentation of late effects of children and adolescents mandatory in clinical pratice to assess the outcomes of therapeutic modalities and manage these late effects. **The understanding of the determinants of this late morbidity is essential to improve future patients' life quality and expectancy by developing risk-adapted treatment and surveillance strategies**.

1.1.2 Endocrine Dysfunctions

Endocrine dysfunction is the most common long-term effect of RT, and can also be present prior to the start of radiotherapy due to damages caused by the primary tumour or surgery. The growth and development of children are subject to complex hormonal regulation by the pituitary gland and the hypothalamus in the CNS. Depending on the dose of radiation, irradiation of the head and neck area can damage the hypothalamo-pituitary function and thus result in hormonal dysfunctions, including deficiencies of growth hormone (GH), sexual hormones, luteinizing hormone (LH), follicle stimulating hormone (FSH), as thyroid stimulating hormone (TSH) or adrenocorticotropic hormone (ACTH). Hormonal dysfunctions can have serious consequences for the further development, especially in childhood and adolescence. Many hormonal deficits develop slowly and the clinical signs are only apparent at a late stage. Late effects of hormonal dysfunction or hypofunction after cranial irradiation include microsomia, missing or delayed puberty, fatigue, adynamia, and potentially life-threatening crises like hypocortisolism. Growth hormone deficiency and pubertal disorders also lead to osteoporosis in many cases. The somatotropic axis is particularly susceptible to damage by radiotherapy. A development of growth hormone deficiency after cranial irradiation is still observed years after initial treatment [13-15]. However, it is not exactly known if low-dose exposure of the pituitary and hypothalamic region leads to damages and which temporal latencies are relevant as organ doses to these structures could only be roughly estimated with earlier techniques.

HARMONIC will assess the association between dose-volume parameters and endocrine dysfunctions, with a main focus on GH deficiency and hypothyroidism, after photon or proton beam therapy. It will quantify the dose and volume-effects of radiation exposures to the pituitary and hypothalamic structures as well as on the thyroid gland in a subgroup of patients who received proton or photon

therapy in the CNS, head and neck region, upper thoracic aperture or cervical spine. Modifying factors such as age at exposure, genetic predispositions, comorbidities and cancer and non-cancer medications that potentially underlie differences in individual radiosensitivity for endocrine dysfunction will also be investigated.

1.1.3 Cardiovascular Toxicities

Cardiovascular disease is the most frequent non-neoplastic cause of death in childhood and adolescent cancer survivors, and mortality from cardiac causes remains higher in these patients compared to the general population [16-18]. Childhood and adolescent cancer survivors have a high risk of symptomatic cardiac events at an early age, and this risk remains elevated for at least 30 years after treatment, wherein almost one in eight will have a severe heart disease. Long term cardiotoxicity can manifest as myocardial ischemia, left ventricular dysfunction, heart failure, valvular disease, pericardial disease, or arrhythmias [19-21]. The role of irradiated heart volume remains unclear. The consequences of irradiating a large part of the heart with a lower dose versus irradiating a smaller part of the heart with a high dose have recently started to be studied [22, 23]. Indeed, a recent study reported a significantly elevated risk of cardiac disease after cancer treatment even when <10% of the left ventricle volume received >30 Gy [22]. The radiation dose–volume effects in the heart and cardiac substructures have been explored only in a few studies. The mean dose to the heart provides an incomplete picture of the risk of cardiac diseases as small volumes of the heart could receive highly inhomogeneous doses. The evaluation of the effect of radiation doses received by the cardiac substructures (eg. heart valves, pericardium) is also crucial for understanding the biological mechanisms leading to specific cardiac toxicities.

HARMONIC will investigate the association of radiation exposure factors to the heart and cardiac substructures with early biomarkers of cardiac dysfunction such as B-type natriuretic peptide (BNP), creatine phosphokinase (CPK) and troponin as well as echography markers such as ejection fraction or fractional shortening.

1.1.4 Neurovascular Damages

Neurovascular late effects after radiotherapy for childhood brain tumours are not very well described in the literature [24]. Cerebral microbleeds, cerebral cavernous malformations and white matter lesions are recognized as a sign of late small vessel disease. Clinically this can lead to neurocognitive dysfunction, dizziness, headaches and other neurological symptoms. Long-term survivors of pediatric brain tumours are also at an increased risk of cerebrovascular accidents compared to the general population due to large vessel artheropathy [25-27]. There has been proposed a relationship between the dose to specific neurovascular structures and the risk of developing a cerebrovascular accident [26-28]. In HARMONIC, we will investigate and validate the extent and risk factors related to neurovascular events, quantify radiation dose-volume relationships on neurovascular structures for development of neurovascular pathologies, and explore imaging changes as a precursor for neurovascular events.

1.1.5 Second Primary Cancers

Second primary cancers are well-known possible adverse effects of radiotherapy, especially in long-term cancer survivors. Normal tissue radiation exposure is associated with increased risks of many solid cancer sites (CNS, thyroid, breast, lung, gastrointestinal organs and tracts, soft tissues and bones), leukemia and myeolodysplasia (and possibly non-Hodgkin lymphoma) over a wide dose range (less than 100 mGy to more than 30 Gy), which typically occur years to decades after exposure [29-31].

Young patients are specifically prone to develop second primary cancers, for several reasons:

- The long life expectancy of many childhood and adolescent cancer survivors is compatible with the typically long latency time of radiation-related cancer incidence. Moreover, while rela-

tive risks for many cancer sites decrease over time, the absolute risks of developing a radiation-induced cancer increases when young patients become older due to a sharp increase in the baseline risk from age 50-60 and multiplicative interactions between risk factors for cancer [30, 31];

- For a given radiation dose, childhood and adolescents are more susceptible to develop radiation-related cancers than adults [29-32]. There is strong evidence of an increased risk per Gy with decreasing age at exposure for leukemia (other than chronic lymphocytic leukemia), breast, brain and thyroid cancers, and moderate evidence for other cancer sites such as non-melanoma skin, bladder and stomach cancers [32]. This higher susceptibility to ionizing radiation relates to a number of developpemental and physiological differences between children, adolescents and adults. In addition, a larger proportion of pediatric tumors (8.5% in a large case serie [33]) compared to adult tumors have germline mutations, and some of those mutations are associated with an increased sensitivity to ionizing radiation or DNA repair deficiencies;
- In radiotherapy (as with other medical procedures using ionizing radiation), whole-body radiation exposure is higher for smaller patients due to leakage irradiation from the head of the accelerator and collimators [34].

Subsequently, second primary cancers have been relatively frequent among long-term childhood and adolescent cancer survivors – overall, 20% of those who were treated in the 1970-80's developed a second cancer by age 50 [5]. Children and adolescents treated with cranial radiotherapy had a 10 times higher risk to develop a subsequent CNS tumor compared to their siblings [35]. Among females who were treated with chest radiotherapy before the age of 21 years, the cumulative incidence of breast cancer was 30% [36] - a rate that is comparable to that of BRCA mutation carriers in the general population, and requires lifelong surveillance.

The shape of the cumulative radiation dose-risk relationship is relatively well described [29], even though the risk estimates are less robust at the lowest (<1 Gy) and highest (>30 Gy) doses, due to small excess risks that are difficult to detect (at low doses) or small case numbers (at high doses). However, the effects of dose fractionation [37], normal tissue irradiated volumes and dose gradients [38], and beam qualities (see Section 1.2.6) have been very poorly described, even though they are both biologically plausible, which makes attempts for risk prediction in treatment planning or replanning quite uncertain. Up to now, the impact of modern EBRT techniques on second primary cancer risks has not been quantified in clinical studies (see Section 1.2.6).

Increased risks of subsequent acute myeloid leukaemia, myelodysplastic syndrome and some solid cancers (i.e. lung, thyroid, gastrointestinal, bladder, endometrial cancers, and sarcoma) have also been reported in relation to several chemotherapeutic agents such as alkylating agents, topoisomerase II inhibitors and antimetabolites, as well as in relation to hormone therapy [39]. However, more research is needed to fully characterize the effect of specific drugs and new agents, and their combined effects with radiotherapy, in the context of multidrug regimens and multimodal treatment strategies. **To improve our understanding of individual susceptibility to cancer treatment-related second primary cancers, it is important to assess the combined effect of radiation factors (i.e. total dose, dose fractionation, volume, beam quality), systemic cancer treatments, and other clinical (e.g. comorbidities, non-cancer medications), lifestyle (e.g. smoking), and hormonal (e.g. puberty) factors**. Based on detailed data on radiation characteristics and all other above-mentionned factors, measured biomarkers of carcinogenesis (see Section 1.3.3), and a long-term, standardized follow-up of patients with passive methods, HARMONIC will provide direct evidence for modern EBRT techniques, and improve our understanding of the determinants of second primary cancer risks, and factors underlying individual susceptibility to cancer treatment-related risks. This new evidence should help refining risk prediction models used for radiation treatment plan optimization and long-term surveillance strategies.

1.1.6 Quality of Life, Educational and Social Outcomes

Given the progress in overall survival after childhood cancer, a critical issue of modern pediatric oncology is to reduce the morbidity burden of treatment, without compromising chances of survival. Such a burden can be qualified objectively with measures of health-related quality of life (HRQoL). HRQoL is an informative indicator of overall health because it captures information on the physical and mental health status of individuals and thus provides a **comprehensive assessment of the burden of a given disease and treatment**. In addition, **economic evaluation** and the computing of quality-adjusted life years based on HRQoL measures can provide a useful comparison of the costs and impacts of different treatment strategies [40].

HRQoL of patients treated with protons has been poorly described, with most studies including small numbers of patients (ranging from 10 to 140) in single institutions (e.g. [41-44]). All the studies indicated an increase in HRQoL scores with time since treatment. After five years of follow-up, HRQoL scores were even identical or above those of healthy children in two studies [41, 42].

HARMONIC involves prospective patient recruitment, with patient contact maintained through their clinicians. In the context of prospective recruitment of patients with direct contact through clinical oncologists, our project fully integrates, as outcome of interest, the evaluation of HRQoL of paediatric patients treated with ionising radiation. We will investigate, through questionnaires, medium- and long-term HRQoL, as well as social and academic outcomes in paediatric patients treated with modern EBRT techniques, and identify clinical and socioeconomic determinants.

1.2 Expected Benefits of Modern Radiotherapy Techniques to Improve Late Health and Social Outcomes

While the use of radiotherapy has been reduced in the past years for management of low grade tumors, it remains a key component of multimodal treatment strategies for many cancer patients, including children and adolescents. Currently, external beam radiotherapy technique (EBRT) is used to treat about 50% of children and adolescents with cancer. Brachytherapy can offer considerable advantages for some patients to reduce long-term treatment sequelae while achieving good tumor control rates, but it is not frequently used in children and adolescents. Stereotactic radiotherapy is also very unfrequently used in paediatrics. The following sections thus focus on conventionally fractionated EBRT.

1.2.1 Overview of Recent Advances in EBRT

Radiotherapy has been routinely administered in paediatric oncology since the 1930s. After orthovoltage and cobalt therapy, photon-beam radiotherapy has been continuously developed over time, and the use of two-dimensional techniques was replaced by 3D conventional techniques in the 1980s, and then by 3D conformal radiotherapy (3DCRT) in the 1990s, which is now treatment standard.

Over the last two decades, considerable technical progresses have been made in radiotherapy [45, 46]. These progresses were achieved thanks to advances in imaging, which allow better delineation of the target volume as well as taking into account anatomical variations and organ motions in treatment planning, and the development of new irradiation techniques such as intensity-modulated radiotherapy (IMRT) and proton beam therapy (PBT), which allow higher dose conformality to the target volume. Compared with conventional or 3DCRT, these advanced techniques increase the dose gradient between the target volume and normal tissues located near the treated tumor, which allows to better spare normal tissues surrounding the treated tumor while delivering a more homogeneous dose to the target volume.

3DCRT uses information from computed tomography (CT) to visualise the tumour to be treated as well as surrounding organs at risk that should be spared. 3DCRT forms the radiation beams to fit the size and shape of the tumour. However, this type of EBRT is administered in a robust fashion, usually with approximately three or four fields and a uniform dose in each field. Therefore, a large volume of surrounding normal tissue may still receive a significant proportion of the prescribed dose.

With **IMRT**, multiple photon beams are aimed from different directions and with modulated intensities, allowing improved conformity, better sparing of surrounding normal tissues, dose painting and dose escalation, as compared with 3DCRT. IMRT thus has features that could make it particularly interesting for the irradiation of pediatric patients who are prone to develop late toxicities. However, the effort of clinicians in planning and quality assurance is higher using IMRT and the duration of treatment per session increases. Thus, IMRT is more costly than 3DCRT. Another downside of IMRT is an increased volume of normal, distant tissues irradiated at low- to moderate dose (see Section 1.2.2). A specialised version of IMRT is **volumetric modulated arc therapy** (VMAT). Instead of using multiple fields to treat the tumour, the linear accelerator rotates around the patient, irradiating the tumour continuously. VMAT is therefore faster than IMRT, which may increase treatment capacity and reduce waiting times, and even more conformal because the target volume is irradiated from infinite positions, thereby giving an even lower but more spread-out doses to surrounding and distant normal tissues.

The latest advance in radiotherapy is **PBT**. Compared to photons (used in 3DCRT and IMRT), **protons** have specific physical properties providing clear dosimetric advantages to improve treatment conformality and reduce doses to normal tissues. The beam delivery of protons is currently carried out in either traditional passive scattering or more modern active scanning mode. For scattered techniques, patient-individual hardware helping to adjust the proton beam to the tumour volume needs to be manufactured. However, in tumours with extensive distal volume, **passively scattering delivery techniques** may be disadvantageous regarding tissue proximal to the tumour. **Active scanning techniques** are based on the magnetic steering of single beamlets. As the target volume is irradiated layer by layer, typically no apertures are required. Scanning techniques can achieve even more conformal coverage of target volumes in some tumours and enable **intensity-modulated proton therapy** (IMPT). IMPT, which is considered to be the most conformal and modern form of PBT, offers the opportunity to optimise dose distribution by using computer-assisted methods that enable the best possible dose application to tumour tissue while at the same time protecting the normal tissue as much as possible.

Last, all types of radiotherapy (both photon and proton beam) can now be adapted during the course of treatment (**adaptive, or image-guided radiotherapy**). During the course of radiation, repeated CT scans are taken to visualise inter- and intra-fractional changes in patient anatomy and tumour volume, which allows adapting the treatment plan and take these changes into account in dose calculation and patient repositioning.

1.2.2 Dosimetric specifics of modern EBRT techniques

Dosimetric studies consistenly reported higher dose conformality with IMRT or PBT vs. 3DCRT (and with PBT vs. IMRT for some treatment plans and organs at risk [47]), with significant dose reductions to organs at risk located within or near the target volume [46].

Despite of **indisputable dosimetric advantages of modern EBRT techniques to reduce high-dose irradiation to normal tissues surrounding the target volume,** these techniques also have **pitfalls and limitations**. With IMRT (and VMAT), the dose reduction to surrounding organs at risk in comparison with 3DCRT is at the expense of larger volumes of out-of-field tissues receiving low-to-moderate doses from **collimator scatter and head leakage irradiation** [48, 49]. Several simulation studies reported that the subsequent increased whole-body radiation dose might lead to an increased risk of second primary cancers [34, 50].

The dosimetric advantages of protons over photons are, at first, a reduced lateral dose scattering. Secondly, while the peak dose deposition with photons occurs shortly after entering the tissue and then decreases continuously until the exit of the body, protons‘ energy loss increases with decreasing pace, resulting in a fairly constant low dose at the entry region and a steep fall-off of energy (called the Bragg peak) when the beam stops. Protons thus have very interesting physical proprieties that allow depositing the highest dose to the tumor and steeply reducing the dose to organs at risk located near the treated tumor. However, there are potential disavantages with PBT [51]:

- In particularly complex treatment plans, **Bragg peak placement inaccuracy** is possible in some cases due to heterogeneous tissue densities, patient movement, daily positioning, and beam delivery-related factors, such as lateral scattering of the beam;
- Within Bragg peaks, energy is deposited as clustered rather than sparse ionisation events, resulting in **complex DNA damages** which are more difficult or impossible to repair and enhanced biological effects. These may be advantageous within a tumor, but possibly deleterious for normal tissues in the tumour vicinity which may receive the full or near-full prescribed dose to achieve local tumour control;
- Currently, the medical prescription of PBT dose includes a 10% reduction in dose to all tumours and tissues to compensate for an enhanced **relative biological effectiveness (RBE) of protons** vs. photons. A RBE value of 1.1 is consistent with numerous experiments and clinical observations on cell survival and tumor control [52, 53]. However, physical experiments have demonstrated that the RBE of protons is not constant all along the Bragg peak but steeply increases at the Bragg peak fall-off, resulting in an increased RBE at the border of the treated volume. The RBE values of protons for endpoints other than cell survival and tumor control, which are relevant **for normal tissue toxicities**, nevertheless remain uncertain;
- PBT is associated with an increase of **whole-body secondary neutron doses** as compared to photon irradiation, which are mainly due to interactions between protons and the metal apertures but also depends on beam quality and incidence and the treatment machine [54-56]. Several simulation studies (but not all) have reported a possible increase in risks of second primary cancers resulting from secondary neutron doses when passively scattering delivery techniques are used [34, 57]. On the opposite, active scanning PBT decreases the whole-body exposures compared to IMRT and 3DCRT [50].

In EBRT, both for protons and photons, kV-imaging is still used at multiples stages of the treatment procedure: for diagnostic, treatment planning or during individual treatment session for adaptive or image-guided radiotherapy. If treatment planning nearly exclusively relies on Computed Tomography (CT), adaptive / image-guided radiotherapy practices vary in imaging frequency and may use either cone beam CT volumetric or planar imaging. Cumulative imaging doses in EBRT are difficult to estimate as they result from mutiple image acquisitions. In addition, imaging protocols highly depend on the pathology and patient morphology, which is associated with wide dose ranges. Based on diagnostic reference levels [58], the doses per imaging exam are estimated to range between 10 mGy for chest scan and 60 mGy for head scan. For image-guided radiotherapy, a single kV-CBCT delivers doses (in soft tissues) in the 10-90 mGy range while doses coming from planar imaging systems are in the 1 mGy range (per image pair) [59]. Overall, when considering the worst case scenario, with a kV-CBCT at every treatment fraction, total imaging doses can reach a 1-4 Gy range. Last, the high technical complexity of the EBRT modern techniques require highly qualified and experienced human resources for treatment planning and delivery, and expensive equipments which may increased waiting times to radiotherapy.

1.2.3 Dissemination of recent advances in EBRT in clinical practice

Recent advances in radiotherapy techniques have been rapidely and widely disseminated in routine practice, including in pediatrics. Since the early 2000s, IMRT has become a standard technique beside 3DCRT to treat paediatric tumours, being available in most European radiotherapy centres [60]. For instance, in 2016, IMRT was used in 90% of the French radiotherapy centers to treat a total of 30 000 patients, versus 20% in 2009 [61].

The use of PBT still remains limited due to the important financial and human resources needed, but the demonstrated dosimetric advantages of this technique have led to a very rapid expansion of its use in high-income countries. In Europe, eight proton centers were in operation in 2010[2]. By 2025, 38 centres are expected to be in operation. Worldwide, the number of centers is expected to rise from 29 in 2010 to 133 in 2025. It is currently estimated that 10% to 30%, depending on the country, of chil-

[2] PTCOG website, https://www.ptcog.ch/ accessed on May 4th 2017

dren and adolescents receiving radiotherapy in Europe, Japan and the United States are treated with protons [62].

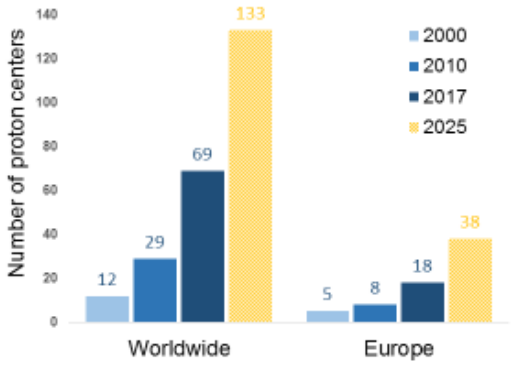


Figure 3. The growing use of protons in Europe and worldwide between 2000 and 2025 (source: PTCOG website, https://www.ptcog.ch/ accessed on May 4th 2017)

1.2.4 Current indications in children and adolescents

At the present time, EBRT is used to treat pediatric patients with tumours of the CNS, neuroblastoma, lymphoma and soft-tissue and bone sarcoma. 3DCRT, IMRT and PBT are internationally accepted as standard techniques for EBRT. Therefore, in several national guidelines, all pediatric tumors are potentially eligible for PBT, whereas some countries have defined specific indications for protons, with those tumor types being referred to PBT centers [63]. There is a large consensus to consider the following tumor types as indications for PBT: medulloblastoma, ependymoma, low-grade glioma, intracranial germ cell trumors, craniopharyngioma, atypical teratoid rhabdoid tumor, and retinoblastoma [62, 64]. In addition, chordomas, chondrosarcomas and parameningeal, orbital, spina/paraspinal and pelvic sarcomas are typical candidates for PBT. General factors to prioritize a patient for PBT are usually very young age, a good prognosis, a larger target volume, and critical organ at risk in close proximity to the target (e.g. the heart, cochlea, hypothalamus, and pituitary gland). As PBT techniques have overcome most of the previous technical restrictions like organ motions, even more indications are emerging, such as Hodgkin lymphoma and neuroblastoma, with the aim of reducing the risk of cardiac, renal and lung toxicities. For total body irradiation, whole-abdomen irradiation, whole-lung irradiation, and whole-brain irradiation, 3DCRT and IMRT are the recommended techniques.

1.2.5 Factors related to the choice of EBRT technique for pediatric patients

While IMRT is now available in most radiotherapy centers of high-income countries and the number of PBT centers is rapidly expanding, the capabilities for PBT remain limited mainly due to higher costs. Therefore, in daily practice, patients who are referred to PBT centers are selected based on their age, prognosis (palliative cases are usually not refered to PBT centers) and tumour localization which are associated with the likelihood of developping treatment-related toxicities-. The tumor site is a very important criterion for selecting an optimal technique, when critical surrounding tissues must be spared. However, patient selection also depends on healthcare organizational factors, i.e. access and referral capabilities (including travel burden for the families), capabilities to ensure effective and timely multimodal therapy, incentives or barriers for collaboration between referral pediatric oncology departments and specialized radiotherapy centers. In the United States, patient/parents‘ socioeconomic status and geographical distance between the place of residence and PBT facility have also been reported to be associated with the likelihood to receive PBT [65].

1.2.6 Late Outcomes of Modern EBRT Techniques

Numerous dosimetric studies support the theoretical advantages of IMRT over 3DCRT, and PBT over IMRT to reduce surrounding normal tissue toxicities. However, **clinical evidence on the efficacy and effectiveness of modern EBRT techniques remains limited**. No randomized trial has been conducted to evaluate IMRT or PBT in paediatric patients, and very few have been conducted, or are

ongoing in adults. Most of the available data in paediatrics come from single-arm prospective and non-comparative, small-sized retrospective studies, and very few studies have compared outcomes of different EBRT techniques.

While IMRT has now been used routinely for almost two decades, little evidence from observational studies has been accumulated so far on late toxicities among children and adolescents. In a (unpublished) systematic review we conducted to summarize all reports published up to 5 March 2019, we found only 17 studies with ≥30 patients reporting late toxicities after IMRT in children and adolescents for few indications (mainly nasopharyngeal carcinoma, medulloblastoma, ependymoma, other intracranial tumors, and rhabdomyosarcoma). These studies involved small patient numbers, had median follow-up times less than 10 years, and mostly had no comparative group. The relevance of predictive (simulation) studies on IMRT, which are based on dose-risk models for late toxicities derived from large retrospective cohorts of childhood and adolescent cancer survivors treated in the 1940-1990s, also remains uncertain. While IMRT is associated with higher dose gradients and larger low-to-moderate dose volumes than past EBRT techniques, previous studies did not assess the effect of the irradiated normal tissue volume *per se* and dose-volume distributions due to a limited dosimetry and/or inadequate analytical methods [38]. Yet beyond cellular effects, tissue effects (through kinetic behaviours of the cell population, radiation-induced genomic instability, bystander and abscopal effects, and stem cell repopulation) may influence DNA damage and repair mechanisms, and drive volume effects of radiation exposures. Several studies on late cardiac toxicity have considered the dose-volume distribution to the heart or vessels, with little evidence of volume effects [66]. These results were, however, related to highly standardized treatment plans with low individual variability, and it is uncertain whether they can predict risks in patients today who receive individualized treatments with highly conformal techniques.

Unlike IMRT, there have been numerous clinical studies reporting PBT outcomes over the past decade [67, 68]. However, most of them were single-institution studies with a relatively small sample size, non-standardized methods of follow-up and a too short duration of follow-up to fully characterize the extent of clinical benefits and the long-term effectiveness of PBT for various indications in children and adolescents. Preliminary reports indicate reduced risks of endocrinopathies [15, 69, 70] and neurocognitive impairments [71], but no significant changes in risks of ototoxicity, visual loss and vasculopathy with PBT compared with photon beam radiotherapy for CNS tumors [72-74]. One study reported better HRQoL scores in patients treated with PBT than in patients treated with photons, but the two comparative case series differed with respect to race, cancer diagnoses and radiation dose [43]. Craniospinal PBT has been reported to be associated with more frequent and severe early MRI changes than IMRT, which may suggest an increased RBE for specific normal tissue toxicities (see Section 2.2.1). However, these results are difficult to interpret due to the lack of dose-volume data to the observed lesions and differences in times between EBRT, surgery and chemotherapy between the two patient groups [75]. In addition, these results were reported for small case series and did not correlate with clinical symptoms. Last, whether the increase in secondary neutrons doses with PBT (as mentioned in Section 2.2.1) translates into increased risks of second primary cancers remains unproved. Studies that have reported so far data on this outcome were fully inadequate in terms of patient numbers and duration of follow-up to assess any increase or reduction of second cancer risks with PBT compared to modern photon beam techniques [76, 77].

Overall, **dose-volume constraints specifically adapted to paediatrics are lacking, both for photon and proton beam radiotherapy**. There have been large efforts to summarize available data on risk for acute and late toxicities and reach consensus for defining dose-volume contraints in treatment planning for adult patients [66, 78]. However, this information does not necessarily apply to children and adolescents who are more likely to develop long-term adverse outcomes than adults because they are typically more vulnerable to radiation exposures. In addition, children and adolescents develop different long-term toxicities than adults because the typical body sites affected by childhood and adolescent cancers lead to normal tissue irradiation of somewhat different organs compared with adults, and most young patients have a long life expectancy and may develop treatment-induced se-

cond cancers and severe injury decades after treatment. **Improving treatment plan optimization and patient selection for highly conformal EBRT techniques in the context of constrained resources thus require accumulating long-time data specifically for paediatrics**.

To facilitate multinational and interdisciplinary collaborations, achieve sufficient sample sizes to study rare diseases such as childhood and adolescent cancers, and promote high data quality standards, the HARMONIC project is setting-up an European registry of paediatric patients treated with modern EBRT techniques, with standardized data collection and methods for exposure and outcome assessment.

1.2.7 Gaps in knowledge

The HARMONIC project will contribute to fill important gaps in knowledge about on long-term outcomes of radiotherapy (and systemic cancer treatments) in paediatrics, with the ultime goal of improving cancer care in children, adolescents and young adults:

1. **Late outcomes, including HRQoL, and effectiveness of advances in EBRT;**
2. **Effect of normal tissue dose-volume distributions and dose fractionation on long-term adverse outcomes;**
3. **RBE of protons and secondary neutrons for late adverse outcomes;**
4. **Correlation of early- and intermediate-term blood/saliva and imaging markers with long-term clinical findings; identification of biological and radiological precursors of long-term, clinically significant adverse outcomes;**
5. **Interactions between radiation, surgery and systemic cancer treatments, and effect of time between different cancer treatment modalities on late adverse outcomes;**
6. **Determinants of individual susceptibility to late adverse outcomes related to cancer treatments;**
7. **Paediatric-specific dose-volume constraints for reduction of endocrine toxicities in photon or proton beam radiotherapy;**
8. **Paediatric-specific dose-volume constraints for reduction of neurovascular toxicities in photon or proton beam radiotherapy;**
9. **Paediatric-specific dose-volume constraints for reduction of cardiovascular toxicities in photon or proton beam radiotherapy;**
10. **Paediatric-specific dose-volume constraints for reduction of second primary cancer risks in photon or proton beam radiotherapy;**
11. **Determinants of early-, intermediate-, and long-term HRQoL after radiotherapy in contemporary populations of children and adolescents with cancer.**

1.3 Methodological considerations

1.3.1 Need for International Collaborations

Childhood and adolescent cancers are **rare diseases** occurring in about one in 500 individuals overall. Subsequently, **low volumes of paediatric patients are usually treated in radiotherapy centers**, in both countries with a large number of facilities (e.g. France, Germany) and countries with a centralized organization of care in pediatric oncology which typically have small population sizes (e.g. Denmark, the Netherlands). For instance, an average of 30 paediatric patients is treated in PBT centres each year [62]. Childhood and adolescent cancers are also **heterogeneous diseases**, with a variety of histologies and locations. Their management thus involves **various therapeutic strategies**, including different irradiation protocols and drugs in combination or not of surgery, in the context of a rapid dissemination of technical advances particularly in radiotherapy.

The rareness of these diseases and the variety of related multimodal treatment strategies require that international and interdisciplinary efforts are set up to evaluate the efficacy and effectiveness of cancer treatments for particular clinical indications with sufficient sample sizes [79-81]. The **current variabil-**

ity of practices [62] highlights the need of improving consensus on optimal EBRT techniques for specific indications and standardizing treatment planning in paediatric patients.

Following previous EU-funded projects, namely the ALLEGRO project (FP7-EURATOM-FISSION n°231965, 2009-2011) on early and late health risks to normal tissues from the use of existing and emerging techniques for radiotherapy and the ANDANTE project (FP7-EURATOM-FISSION n°295970, 2012-2015) which demonstrated the feasibility of reconstruction of dose to out-of-field organs for PBT, HARMONIC serves as a pilot for a future long-time pan-European registry of particle and photon beam therapy in children and adolescents to promote collaborative and interdiscisiplinary research activities in Europe. In particular, while access to PBT remains limited (see Section 1.2.3), even for those tumours most effectively treated with protons and the range of applications of PBT is still being explored (see Section 1.2.4), the timing is thus ideal to launch such an initiative in Europe to provide early detailed evidence on the late outcomes of protons in paediatric patients.

1.3.2 Individual Dosimetry for Modern Radiotherapy Techniques

Treatment planning systems (TPSs) are conceived to compute an accurate dose distribution in the irradiated field in a time acceptable for the routine clinical practice. This time limitation imposed on TPSs, which is of the order of some minutes, translates into the introduction of approximations in the algorithms employed for the dose computation. These approximations are, in general, valid for absorbed dose distributions computed inside the irradiated volume, and to some extend, near it. Even the so-called Monte Carlo TPSs use some approximations. TPSs, both for photon and proton therapy, rely on some sort of virtual source model which produces particles according to the energy and angular distributions that characterize the beam. A virtual source model is conceived and fitted to reproduce the fluence spectra of the beam. By its very nature a virtual source model is not tailored to reproduce absorbed dose distributions outside of the irradiated field, therefore, scattered and contaminant particles far-from-the-field are not taken into account, hence limiting the validity of absorbed doses computed in the regions of interest for the estimation of second cancer probabilities. These problems can be surmounted by means of the Monte Carlo simulation of the geometry of the collimating and scattering structures around the beam path in the gantry, both for photon and proton therapy facilities. To follow this approach general-purpose Monte Carlo codes, such as PENELOPE (photon therapy) or Geant4 (photon and proton therapy), are required. These codes allow to tally phase-space files that accurately represent the beam and that can be subsequently used for the computation of absorbed dose distributions far from the irradiated field. Geant4 also simulates secondary neutron production, essential for the computation of the absorbed dose in low dose regions when photon or proton beams with energies higher than 10 MV are employed. In epidemiological studies, to overcome the extensive calculation times needed for Monte Carlo simulations, out-of-field doses from different scatter contributions can be estimated with analytical models based on a computational human phantom matched to the patient structures acquired for treatment planning (with CT and/or MRI).

Absorbed doses coming from imaging system are not computed by the TPSs in clinical routine. Therefore, they also have to be computed by Monte Carlo in the same manner as for out-of-field treatment doses calculation. Depending on the level of details known for a given imaging system, virtual source model [2] can be used in lieu of full space phase file [82].

1.3.3 Biomarkers of radiation-induced lesions

A biomarker has been defined as “any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical or biological”. Biomarkers might be used for 1) estimation or validation of received dose; 2) investigation of individual sensitivity/susceptibility and 3) early detection of radiation-induced health effects. Currently, there is no diagnostic assay available that can reliably predict the risk of adverse health effects [83, 84].

In parallel with the effects of dose per fraction, total dose and beam quality and organs at risk, previous studies showed that there are several important mechanisms affecting adverse health effects [85, 86]. There is strong evidence that elevated oxidative stress and related response pathways, inflamma-

tion, DNA repair and damage response are correlated to the risk of ionizing radiation-induced healthy tissue damage [85]. The use of biomarkers for prediction of long-term effects and individual susceptibility is a promising new technology in radiation risk research. However, before a biomarker is tested on an epidemiological cohort, it should be verified in a defined small group of patients with accurate and detailed dosimetry, so that potential confounding factors can be eliminated as planned in HARMONIC. In the project, blood, saliva, and echography and MRI images are collected from patients treated with EBRT whose particular organ at risk is included in the radiation beam such as large and small vessels in brain and thorax where radiation induced vascular damage is frequently observed.

The novel approach of HARMONIC is the adoption of a multidimensional approach, where changes induced by radiation are assessed at the level of the transcriptome, proteome (plasma and saliva proteome profiling) and epigenome (protein modifications) as well as inflammation and oxidative stress levels and on radiological images [83, 84, 86-88]. This approach will not only allow identifying a fingerprint of exposure, but will also yield new mechanistic insights into the action of low dose radiation on small number of patients that is indispensable for future studies on biomarkers. While the most serious health effects will develop more than five years after radiation exposure, HARMONIC investigates early changes of potential biomarkers that may provide information about the activation/deactivation of biological processes which may be involved in the development of the late adverse health effects. In parallel, new mechanisms are explored using cutting edge techniques available. HARMONIC develops and implements appropriate bioinformatical methodologies for validation of biomarkers in blood and saliva samples, and novel bioinformatical approaches for integration of results considering dose, volume and beam quality. The feasability of use of saliva sampling as a non-invasive, relatively simple and cost-effective collection method for future large-scale epidemiological studies is also assessed [89].

Protein activation

Long-term toxicity of radiotherapy can be observed on large vessels, causing cerebrovascular diseases [26, 90] or leading to ischemic cardiac disease [20, 91]. Radiation may cause microvascular damage and decreased blood flow, induced death of cardiomyocytes and their replacement by collagen and fibrous tissue, which can lead to myocardial dysfunction and thus cause arrhythmias and conduction disorders. Radiation can also cause vascular damage through different mechanisms, e.g, interacting with the pathological pathway related to premature ageing processes, such as atherosclerosis of coronary arteries with the disease observed at younger ages than in the general population. Studies have shown that pro-inflammatory cytokines and chemokines are directly involved in cardiac remodeling e.g. changes in cardiac shape, size and function as a response to injury. Indeed, in experimental models of myocardial infarction, the increase of cytokines of the Tumor Necrosis Factor (TNF) family leads to overexpression of their receptors in cardiomyocytes and induces cardiac remodeling. This induction involves the signaling Rho / ROCK pathway and the nuclear translocation of NF-kB. This leads to the expression of chemokines, such as RANTES and MCP-1 [92]. This loop could contribute to a pathological activation of local tissue. For instance, TGFβ1/Smad and TGFβ1/rho/JNK pathways, other signaling cascades have been linked to fibrosis such as PDGF/PDGFR, IGF/IGFR, EGF/EGFR, TNF-α and FGF-2.

HARMONIC focuses on levels of cardiovascular biomarkers and their activation in relation with radiation therapy and will be performed in close collaboration with WP2, with the use of a reverse phase protein arrays (RPPA) method called NormaCurve, that allows simultaneous quantification and normalization of RPPA data [93].

Inflammation and chronic oxidative stress

Inflammation and chronic oxidative stress are important mechanisms underlying processes of vascular pathologies and cancer [94-96]. In this task, the inflammation processes will be studied using a set of biomarkers e.g. Pentraxin 3 (PTX3), CRP, NF-kB, IL-1 and IL10, and oxidative stress by analysis of 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) in saliva and blood samples. A studies by Björklund and col-

leagues [97] have reported an up-regulation of the cardiovascular disease marker PTX3 in the arteries of patients previously treated with radiotherapy [98]. Their results indicated a sustained increase in vascular inflammation even years after treatment with radiotherapy, which could lead to intimal hyperplasia and vascular occlusions similar to those experienced in atherosclerosis. PTX3 is an acute phase protein, expressed in macrophages, dendritic cells, endothelial cells, fibroblasts and smooth muscle cells [97]. Its expression can be induced by cytokines such as IL-1 and TNF-alpha via NF-kB and JNK pathways respectively. PTX3 is considered as a reliable biomarker for local inflammation in cardiovascular disease, because it is highly expressed in patients with various cardiovascular diseases such as congestive heart failure, angina and acute myocardial infarction.

DNA has been considered to be the most important target for reactive oxygen species as they may result in deleterious changes of genetic information and cellular functions. Among the many types of nucleic acid modifications induced by reactive oxygen species in the oxidative stress situation, 8-oxo-dG has been widely used as a sensitive marker of the general systemic oxidative stress in vivo and in vitro [99]. We have previously shown that the origin of radiation-induced 8-oxo-dG is cellular nucleotide pool [95, 99, 100] where dGTP can be modified by reactive oxygen species to 8-oxo-dGTP, a mutagenic modified nucleotide. An enzyme called MTH1 dephosphorylates 8-oxo-dGTP, which then will be released from cells to extracellular milieu as 8-oxo-dG where it can be determined as biomarker for oxidative stress [99]. It has also been shown that exposure to high, medium and low doses of gamma radiation leads to excess levels of reactive oxygen species and elevated extracellular 8-oxo-dG [101]. It was shown that patients with high levels 8-oxo-dG prior to radiotherapy and with no-change of the levels after radiotherapy have higher levels of severe radiation side effects [102-105]. These findings reveal that the ability to remove 8-oxo-dGTP from the nucleotide pools is an important response for cells following RT and that the inability to achieve this efficiently may lead to elevated levels of early and late side effects. Using salivary and plasma protein profiling as well as studies on protein modifications, new biomarkers of oxidative stress and inflammation processes will also be identified.

HARMONIC will measure 8-oxo-dG levels, inflammatory markers, and plasma protein profiling in blood and saliva [106-109].

Cellular aging and miRNA signature

There is growing evidence that alterations in telomere length and mtDNAcn in blood are important early mechanisms initiating and contributing to cancer risk [110-112]. miRNA dysregulation may be central to the cellular response to radiation exposure, acting as oncogenes or tumor suppressor genes as well as controlling various aspects of cancer biology such as the DNA damage repair mechanisms, apoptosis and cell growth [113]. Additionally, typical circulating miRNAs (e.g. miR-18a, miR-21, miR-155, miR-221, and miR-375) are known to be dysregulated in most cancers, including breast, colon, lung, prostate, pancreas, gastric, ovarian, esophagus and liver [114].

In HARMONIC, genomic DNA is isolated from blood leukocytes and saliva, and telomere length and mtDNAcn are assessed using the RT-qPCR method [86, 88]. In parallel, total RNA extraction is performed and circulating miRNA expression levels are analyzed via qRT-PCR analysis [87, 115].

MRI markers of neurovascular lesions

Vascular damage can occur after radiotherapy for childhood brain tumors. The damage may be divided into small or large vessel disease, according to which part of the vasculature that is affected. MRI changes characteristic for small vessel diseases are microbleeds, lacunar infarcts and white matter hyperintensities seen on Fluid Attenuation Inversion Recovery (FLAIR) sequences. These image changes are similar to them older non irradiated patients and are believed to be caused by chronic ischemic changes to the brain parenchyma. As it is seen in the geriatric population, these changes can be associated with neurocognitive decline. Another aspect of small vessel diseases after radio-

therapy is the formation of cavernomas, which is believed to be cause by neoangiogensis after damage to the small vessels of the brain.

The exact cause of large vessel vasculopathy is not clear, but damage to the vessel walls of the large arteries leading to inflammation and subsequent myointimal proliferation causing thickening of the wall is believed to be a central feature. Damage to the central arteries at the base of the brain, may lead to stenosis or occlusion of these large vessels, in a manner similar to the inflammation characteristic for Moya Moya disease. Clinically patients with large vessel disease may present with transient ischemic attacks, intracranial hemorrhages or ischemic strokes. Mineralizing microangiopathy after radiotherapy can lead to calcifications in the deep structures of the brain, which will be most evident on CT-scans. Frank radionecrosis can also develop after very high-dose radiotherapy to the brain. Although the underlying mechanism is not clear, endothelial damage and the damage to the glial-cells seem to play an important role in this pathology.

2 STUDY OBJECTIVES

2.1 Primary Objectives

The HARMONIC project's main objective is **to evaluate the late health and social outcomes of modern EBRT techniques using photons or protons in paediatric patients**, based on the setting-up of a European, long-time registry complemented by a biobank. More specifically, the research aims:

1. (WP2) To assess the **incidence and severity of late health outcomes, primarily endocrine dysfunctions, cardiovascular toxicities, neurovascular damages, and second primary cancers,** in relation to the dose-volume distribution to non-targeted organs and tissues, radiation delivery technique and beam quality factors, and potential modifying factors (i.e. age at exposure, genetic predispositions, comorbidities, and systemic treatments including chemotherapy, targeted therapy, immunotherapy and hormonal drugs) that may underlie differences in individual susceptibility for these outcomes;
2. (WP2) To assess **social aspects of advances in radiotherapy, primarily HRQoL and academic achievement,** in paediatric patients treated with modern EBRT techniques.

2.2 Secondary Objectives

2.2.1 Scientific objectives

The research also pursues secondary objectives:

1. (WP2) To assess **multidimensional fatigue** in paediatric patients treated with modern EBRT techniques, and identify clinical and therapeutic determinants of fatigue;
2. (WP4) To improve estimation of **patient-specific doses to the whole body and non-targeted organs and substructures** from different radiotherapy delivery techniques (including IMRT and PBT), combining particle transport modelling, experimental measurement, patient images and computational human phantoms to allow whole-body and organ dose estimation for the HARMONIC study participants as well as for future investigations and studies on the optimization of out-of-field doses and dose delivered during imaging procedures. The developed methods will complement and extend the calculations performed by the TPS, and calculate doses from x-ray imaging systems (2D-kV, 3D kV-CBCT, MV-CT) in image-guided radiotherapy;
3. (WP5) To investigate **radiation-induced cellular responses and biological mechanisms related to the occurrence of cardiac and vascular diseases and second primary cancers** in samples of blood and saliva, identify **biomarkers of susceptibility and health effects**, evaluate differences in disease biomarkers in relation to the radiation delivery technique and beam quality factors, and explore the relevance of the use of saliva as a biosampling method

for pediatric cohorts regarding feasibility and the quality and reproducibility for different measured biomarkers**.**

2.2.2 Strategic Objectives

1. To promote **sustained collaborative research activities between the medical and interdisciplinary radiation protection research communities** for improvement of patient care, and inform health care providers and policy makers on the clinical and social impact of advances in radiotherapy in paediatric settings.

2. To serve as a **pilot for a future long-term pan-European registry of children and adolescents treated with particle and photon beam therapy, including a biobank of saliva and blood samples**

The HARMONIC Consortium will develop an infrastructure and instruments for harmonized data collection and patient follow-up for the developpment of a pan-European registry of paediatric patients treated with modern radiotherapy techniques, in partnership with the European Particle Therapy Network (EPTN) – a task force of European SocieTy for Radiotherapy and Oncology (ESTRO) (see Letters of Support in Annex 3). The study is also supported by the Particle Therapy Co-Operative Group (PTCOG), the Paediatric Radiation Oncology Society (PROS), and the International Society of Paediatric Oncology (SIOP). The activities described in the present protocol serve as a pilot for a future long-term pan-European registry of particle and photon beam therapy in children and adolescents, which will be extended from the 5 initial centres (AUH, CRFB, GR, KUL, UK Essen) / 4 countries (Denmark, France, Belgium, Germany) to other radiotherapy centres in European countries that will join the effort in the future. In the Netherlands, PMC and UMCG are already partners of the Consortium to facilitate the extension to the inclusion of Dutch patients. In Spain, two centers (Pozuelo de Alarcón, and Clínica Universitaria de Navarra in Madrid) will start treatments in 2020 with potential inclusion of 20-30 additional paediatric patients per year. Representative members will be invited to the consortium meeting to contribute to the scientific discussions. Integration in the project is envisaged (pending national funding). Extension to other European centers – through medical societies and professional networks of the participating investigators – is also envisioned.

3. To contribute in **future collaborative projects with existing cohorts or registries in Europe**

A number of collaborations with national or European projects or programs are already initiated or envisioned. The HARMONIC Consortium has established a collaboration with the ISIBELa project (Intrinsische Strahlenempfindlichkeit: Identifikation biologischer und epidemiologischer Langzeitfolgen) funded by the German Ministry of Education and Sciences. The ISIBELa project is addressed to the computation of radiation-induced secondary cancer probabilities in adult patients treated several decades ago with conventional radiotherapy, based on data from the German Cancer Registry and computed whole-body dose distributions in anthropomorphic phantoms.

HARMONIC partners (WPE and UK Essen) are also collaborating with other Departments of the University Hospital Essen in a project aiming to evaluate radiation-induced secondary cancers in retinoblastoma patients, who were treated with several EBRT and brachytherapy techniques using x-rays or protons and included in a local follow-up registry. The HARMONIC Consortium's research activities will allow reconstructing the dose distributions delivered to the retinoblastoma patients and computing secondary cancer probabilities.

HARMONIC has initiated a collaboration with the EURADOS network (European Radiation Dosimetry Group), where a task group aims to develop and implement new computational methods for Monte Carlo dose estimation delivered in treatment and imaging procedures, and new epidemiological approaches for the computation of radiation-induced secondary cancer probabilities.

Owing to the relevance of the research objectives of HARMONIC, the Bundesamt für Strahlenschutz (German Federal Office for Radiation Protection) has initiated a collaboration with the Consortium to integrate one of its members into the German Delegation at the United Nations Scientific Committee on the Effects of Atomic Radiation. This incipient collaboration might give the opportunity to present the findings of HARMONIC on radiation-related second primary cancers at the level of the United Nations.

The HARMONIC Consortium also works towards establishing close collaborations with medical societies and professional networks in all countries of the investigating centers, in particular the national childhood cancer registries and paediatric radiation oncology societies or networks.

Within HARMONIC, prospective biosampling (blood and saliva) will be done before radiotherapy, immediately after and at 1 year after treatment. This biobank provides a unique opportunity to study biomarkers of exposure, susceptibility and effects and to validate whether saliva can be used instead of blood for the characterisation of these biomarkers in future large-scale molecular epidemiology studies. The results are planned to form the framework for future investigations identifying and validating predictive biomarkers for cancer risk after exposure to photons or protons as well as promote a better understanding of the mechanisms involved.

4. To contribute in **future international research studies on late outcomes of modern radiotherapy techniques for management of paediatric cancers**

Since paediatric cancers represent heterogeneous diseases, each of them being relatively infrequent, involving specific therapeutic strategies and subsequently different health burden over the patients' lifetime, international research collaborations are necessary to provide robust evidence on late outcomes associated with different treatment plans, and optimal radiation techniques for specific clinical cases. To strengthen research on late outcomes of modern radiotherapy techniques, we seek collaborations with investigators in North America and Asia to set-up multinational research studies [79]. We envision setting up data collection and patient follow-up in HARMONIC in liaison with the U.S. Paediatric Proton/Photon Consortium Registry and Asian centers to enable pooling data for potential collaborative studies in the future. The U.S. registry has included 2,775 patients aged <22 years treated in 15 proton centres as of 25 July 2019 [116]. Since 2018, the registry is also open to photon centers.

3 EXPECTED IMPACTS

HARMONIC WP2 is expected to result in **improved treatment outcomes in paediatric cancer patients**, and more specifically:

- Support **evaluations of the effectiveness and health and social impacts of technical advances** in radiotherapy for paediatric patients;

- Provide **new evidence on the determinants of late health outcomes (including early and intermediate blood and imaging markers of late diseases)** of modern EBRT techniques using photons or protons, and quantity the risks of late outcomes as a function of organ dose-volume distributions, dose fractionation, radiation delivery technique and beam quality factors;

- Support the provision of **new evidence on radiation-induced cellular responses and biological mechanisms that may lead to second primary cancers and cardiac and vascular diseases** after radiotherapy, biomarkers of sensitivity and diseases, and possible modifications in disease biomarkers in relation to the dose-volume distributions, dose fractionation, radiation delivery technique and beam quality factors;

- Support **improvements in individual dosimetry to non-targeted organs for different radiotherapy delivery techniques**, including 3DCRT, IMRT and PBT, and develop novel dosimetry tools to provide the medical community with means to investigate the overall radiation burden, including contribution from CT imaging for therapy planning and re-planning;

- Support **improvements in the recommendations for optimisation of treatment plans in paediatric patients to further reduce late toxicities of radiotherapy**. In particular, the data and results accumulated in the research will complement efforts for harmonized and enhanced guidelines in peadiatrics, such as PENTEC (Pediatric Normal Tissue Effects in the Clinic), which is a volunteer international research collaboration aiming to establish quantitative, evidence-based dose-volume-risk guidelines to inform radiation treatment planning and improve outcomes after radiation therapy for childhood and adolescent cancers [81].

4 ENDPOINTS

4.1 Primary Endpoints

The primary endpoints of the research are incidence and severity of late health and social outcomes of EBRT, which may occur years to decades after treatment, specifically:

- Endocrinopathies
- Cardiovascular diseases
- Neurovascular diseases
- Second and subsequent primary cancers
- HRQoL (physical, emotional, social, and school functioning)
- Academic achievement

4.2 Secondary Endpoints

The secondary endpoints correspond to early- and intermediate-term outcomes of EBRT, which may occur within the first one to five years after treatment, and be predictive of late outcomes:

- Early- and intermediate-term dysfunctions in endocrine hormone levels
- Early- and intermediate-term changes in blood/saliva and imaging markers of cardiovascular diseases
- Early- and intermediate-term changes in imaging markers of neurovascular damages and its relation to the incidence and severity of neurovascular diseases and changes in HRQoL or academic achievement
- Early- and intermediate-term changes in blood/saliva markers of protein activation relating to vascular damages (in relation to late cardio- and neurovascular diseases)
- Early- and intermediate-term changes in blood/saliva markers of oxidative stress and inflammatory response (in relation to late cardio- and neurovascular diseases or second primary cancers)
- Early- and intermediate-term changes in blood/saliva markers of carcinogenesis (in relation to second primary cancers)

The research also allows aims to investigate secondary intermediate-term and late outcomes:

- Multidimensional fatigue (general, sleep/rest, and cognitive fatigue)
- Late morbidity excluding diseases mentioned in Section 4.1
- All-cause and cause-specific mortality

5 STUDY DESIGN

5.1 Type of Study

Multicentric, European, observational cohort of paediatric patients treated with EBRT. The participating centers are listed in Annex 2.

The research does not affect cancer treatment.

5.2 Research Methodology

HARMONIC is an **observational cohort** based on an integrated approach of **conventional epidemiology complemented by non-invasive imaging and molecular epidemiology**, and the development of methods for patient-specific dosimetry for non-targeted organs and substructures to assess long-term health and social outcomes, and inform about the possibilities for optimisation of medical exposures. HARMONIC is based on two complementary methods of constitution, prospective and retrospective.

Prospective inclusion

Prospective inclusion is defined by inclusion before the first administration of EBRT, with patient involvement in the research through blood/saliva sampling, imaging and/or patient and parent-proxy reported questionnaires. In this way, it is possible to collect specific participant's data before and after EBRT. Routine visits of cancer treatment or follow-up (standard care) are the opportunity to perform research procedures, meaning that specific visits to the hospital for research purposes only are not needed. Being noted that patient and parent-proxy reported questionnaires may be completed at home. Interventions are proposed according to the context of treatment (tumor type and/or EBRT field) and are performed on the participant's choice since the interventions are independent from each others.

A **biobank**, including blood and saliva samples, is set up for identification of biomarkers. Saliva sampling is a non-invasive method being particularly useful in children that could lead to increase acceptability and participation of paediatric patients in research, and the possibility of repeated sampling. Saliva sampling will offer the possibility to explore and validate its use as a biosample for molecular epidemiologic studies.

Retrospective inclusion

Retrospective inclusion is defined by inclusion after the first administration of EBRT without patient's involvement (no intervention for research purposes). Retrospective inclusion is performed because the cohort should reflect as much as possible the study population (meaning it allows reducing or minimizing selection biases that might cause false conclusions). The study should be carried out on the entire study population:

- Retrospective inclusion aims to increase the statistical power of the research. By doing this, we can study outcomes in a larger population and with a longer follow-up time;
- Retrospective inclusion includes subjects who died before patient recruitement. It is necessary to include deceased subjects since they have to be part of mortality statistical analyses, and ignoring competing risks of death might cause biased analyses for other outcomes;
- Retrospective inclusion is conducted as necessitated by small patient populations and rarity of late effects over the 10 first years following treatment;
- Retrospective inclusion includes lost to follow-up patients from treatment centres to limit selection biases in the statistical analyses when the follow-up rates are associated with treatment modalities and/or outcomes.

For retrospective and prospective inclusion, all same ***routine data*** from medical records are collected in the participating centers. Routine data is any data from routine care available in medical records.

Detailed individual dosimetry for in-field and out-of-field organs and anatomical substructures based on a dose reconstruction approach will be available for the whole cohort (see Annex 7).

Considering the duration of the project and the post exposure time needed for radiotherapy-related effects on normal tissues to occur, ***linkage to external morbidity/mortality registries and health care databases*** will be performed for passive follow-up. This strategy is a powerfull epidemiologic tool for investigating associations of treatment characterictics with late effects. Passive long-term follow-

up[3] of the study participants through linkage with external registries and databases is also essential to compensate for differential frequency and duration of visits at the treatment centers depending on health status, treatment-related factors and outcomes, socioeconomic and other potential patient-specific factors.

5.3 Research Timeline

Duration of patient inclusion: 10 years, starting in 01 June 2020

Duration of patient participation:

- For blood collection: 1 year (3 visits) after the end of EBRT ;
- For saliva collection: 1 year (3 visits) after the end of EBRT ;
- For cardiac echography: 3 months (1 visit) after the end of thoracic EBRT, or 10 years (5 visits) after craniospinal irradiation (CSI);
- For MRI imaging: 5 years (3 visits) after the end of EBRT; an extended follow-up to 10 years (one 4th visit added) can be possible under further protocol's amendment.
- For HRQoL and fatigue questionnaires: 10 years maximum after cancer diagnostic and without exceeding the age of 25 years.

Duration of long-term passive follow-up: 20 years.
The duration of passive follow-up varies between 10 and 20 years, according to the study participants' date of inclusion. Longitudinal long-term follow-up is essential to assess late toxicities of radiotherapy in paediatrics – an amendment could be considered at the end of this 20-year period to extend the passive follow-up duration.

Total duration of the research (including long-term passive follow-up): 20 years

The study participants are allowed to participate in other research studies at any time.

6 SELECTION OF PARTICIPANTS

6.1 Study Population

In the prospective part, the study population consists of all paediatric patients undergoing EBRT at the participating centers.

In the retrospective part, the study population consists of all paediatric patients who underwent EBRT at the participating centers.

Some endpoints are evaluated in subsets of the study population, for whom specific studied parameters are clinically relevant and technically assessable (see Section X).

6.2 Inclusion Criteria

Table 1. Inclusion criteria for prospective and retrospective inclusion of study participants

Prospective part	Retrospective part
- Scheduled first EBRT (i.e. first fraction of EBRT not already performed) - Age under 18 years at the time of scheduled first EBRT * - Radiation treatment plan stored in DICOM format - Affiliate or beneficiary of health insurance (or any required equivalent as de-	- First EBRT started in 2000 or after (i.e. first fraction of EBRT already performed) - Age under 18 years at the time of first EBRT initiation* - Radiation treatment plan (first EBRT) stored in DICOM format - Basic information on chemotherapy (dates of treatment, drug/protocol

[3] Meaning no participation of the included individuals in the research

fined in applicable national law) - Basic information on chemotherapy (dates of treatment, drug/protocol names) available at baseline, for patients treated with chemotherapy - Usual residency in the country of EBRT to enable a long-term follow-up - Signed informed consent/assent	names) available at the time of first EBRT initiation, for patients treated with chemotherapy - Usual residency in the country of EBRT to enable a long-term follow-up
*under 22 years for France for patients with mediastinal/chest/pulmonary irradiation	

6.3 Non-inclusion Criteria

Table 2. Non-inclusion criteria for prospective and retrospective inclusion of study participants

Prospective part	Retrospective part
- Patients with very poor prognosis (tumor type with typical five-year survival probability <30%), e.g. diffuse pontine glioma or high grade glioma - Prior internal radiation therapy; - Protected adults (persons under curatorship, tutorship / individuals under guardianship by court order, persons deprived of their liberty)* - Adult*/parent(s)/legal representative(s) who cannot read or understand the informed consent in the applicable language(s) in the country of EBRT	- Patients with very poor prognosis (tumor type with typical five-year survival probability <30%), e.g. diffuse pontine glioma or high grade glioma at first EBRT initiation - Prior internal radiation therapy - Patients have expressed a refusal
*for France where the age criteria is until <22 years for patients with mediastinal/chest/pulmonary irradiation	

6.4 Procedures of Recruitment

Study participants are included either retrospectively (after EBRT initiation – from existing paper or electronic records), or prospectively (before EBRT initiation – at the time of the clinic visit for EBRT planning).

According to the hospital records availability in each participating centers and the recruitment period, the total expected number of included patients is **2670 patients** (1910 treated with protons, and 760 patients treated with photons), **by 31 May 2023**[4]. Later, 430 additionnal participants could be included per year. These figures account for a hypothetical overall rate of refusal to participate of 8%, and the following centre-specific information:

- the date of starting photon or proton EBRT of paediatric patients or the date from which DICOM data can be retrieved in the hospital databases, whichever occurs the latest;
- the mean number of patients aged <18 years (or <22 years for France for those with mediastinal/chest/pulmonary irradiation) treated with EBRT per year since 2008 and in most recent years (or anticipated numbers of treated patients for newly operating centres);
- the average %, in recent years, of patients referred from abroad.

Table 3Expected number of study participants recruited by 31 May 2023

Center	Average num-	Retrospective inclusion	Prospective inclusion	Total

[4]The HARMONIC projet has been funded by the European Commision's under the grant agreement No 84770 for a 5-year period, corresponding to retrospective data collection back to 2008 (i.e. the earliest data when DICOM data can be retrievable) and prospective data collection during 3 years (01 June 2020 – 31 May 2023).

	ber of paediatric patients treated per year	Time period	No. patients	Time period	No. patients	
KUL	90	2008-2020	200	2020-2023	230	430
AUH	35	n/a	0	2020-2023	90	90
CRFB	35	n/a	0	2020-2023	90	90
GR	70	2013-2020	380	2020-2023	180	560
UK Essen	200	2013-2020	1140	2020-2023	360	1500
Overall	**430**		**1720**		**950**	**2670**
n/a: not applicable						

7 STUDY PROCEDURES

7.1 Selection

Selection of study participants is established:

- From electronic and paper medical records or hospital databases, and;
- They are identified by physicians at the time of clinic visits at the radiotherapy or paediatric oncology departments of the participating centers where EBRT is performed.

7.2 Inclusion, Information and Approval to Participate

Inclusion is made at the time of clinic visits at the participating centers. The investigator or his/her designated representative shall fully inform on the research during a regular consultation before EBRT, or at initiation of EBRT. The investigator or his/her designated representative will give both oral and written information of all aspects of the research that are relevant to the decision to participate, including that the participation is made with time for reflection, the participation in the research is voluntary and may be declined, and the participation may be withdrawed at any time. Depending on countries, the investigator or his/her designated representative will need to obtain the consent or assent of children and adolescents (Table 4). In addition, parental/guardian(s) permission will be obtained. Child/adolescent and parent(s)/guardian(s) are given the opportunity to ask questions and the investigator or his or her designated representative takes the opportunity to explain anything that is not clear. The information and consent / assent procedures should reflect a reasonable effort to enable the child/ adolescent to understand, to the degree they are capable, what their participation in research would involve. For that, adapted documents on the research description and procedures will be prepared to explain the project to the paediatric patient depending on the age of the subject.

If the child should refuse to participate, his or her refusal takes precedence over parent(s)/guardian(s) permission. The possibility of permission of one parent for the research study will be assessed at each national level. For children who have only one parent(s)/guardian(s), the only signatory must certify that he or she is the only parent(s)/guardian(s).The investigator or his/her designated representative should keep a copy of consent form.The parent(s)/guardian(s) of the minor should be given another copy of both information and consent/assent forms.

When the minor participant reaches the age of majority, a new informed consent for himself or herself is obtained at the following visit, or by regular or electronic mail.The adult patient regular and/or electronic address is asked to parents for the only purpose to send the information and consent forms. In case of contact by regular mail, the adult patient will keep a copy of both information and consent forms and will be asked to send a copy of his/her consent using the included postage-paid envelope to the investigator[5]. In case of contact by e-mail, the adult patient will give his/her electronic consent trough a secured platfom and will receive a copy of his/her consent in his/her electronic mailbox. Reasonable reminders will be made in case of non-response.

[5] Inserm U1018 CESP Cancer and radiation for France

Table 4. Consent and assent requirements for study participants due to national laws and regulation

Country	Consent / assent from study participants		Consent from parent(s)/ guardian(s)
	Legal age of consent	Mandatory or suggested age ranges for assent	Number of required signatories
Belgium	18 years	4 – 11 years 12 – 14 years 14 – 17 years	Both parents or emancipated individuals
Denmark	18 years	15 – 17 years	Both parents or emancipated individuals
France	18 years	4 – 6 years 7 – 11 years 12 – 17 years	Both parents or emancipated individuals
Germany	17 years*	7 – 11 years 12 – 16 years	Both parents or emancipated individuals
*in Germany, both the participant's consent and parental consent are required for adolescents aged 17 years			

Exemption from requirement to provide individual information on data collection for the retrospective part

Since no direct contact with patients is planned if they have already started EBRT or if EBRT is terminated, and given the large size of the cohort, no individual information to patients will be provided regarding the **retrospective data collection** from existing medical records. Additionnaly since retrospective inclusion starts from 2008 and that patients' addresses are not collected, for methodological reason it is not possible to contact former patients and former patients may also include deceased patients.

Linkages to morbidity/mortality and healthcare databases are necessary to provide information on late outcomes under study, and allow minimizing rates of loss of follow-up and differential frequency and duration of follow-up clinic visits depending on the patient's health status, treatment-related factors and outcomes, and/or other potential patient-specific factors. For the same reason than those mentionned above, no individual information will be made.

A collective information on the research will be displayed in the radiotherapy and paediatric oncology departments, including the procedure and contact(s) for study refusal.

7.3 Follow-up

7.3.1 Interventions performed for the research (prospective part)

Interventions are proposed according to the context of treatment (tumor type and/or EBRT field – Table 5), and are carried out at the department that is responsible for follow-up based on already planned visits in regards to standard care. Interventions are performed on the participant's choice since they are independent from each other. Their acceptability by the patients and parents regarding the patient's heath status is at the discretion of the physician who is responsible for patient inclusion.

Table 5. Description of the research procedures to be proposed according to study participants' characteristics

Tumor type (EBRT field)	Age at first EBRT	Interventions/procedures performed for the research
CNS (focal irradiation)	<18 years	• Blood sampling • Saliva sampling • Neurovascular MRI sequences (added to regular follow-up MRI) • Questionnaires

CNS (CSI)	<18 years	• Blood sampling • Saliva sampling • Neurovascular MRI sequences (added to regular follow-up MRI) • Cardiac echography • Questionnaires
H&N	<18 years	• Blood sampling • Saliva sampling • Questionnaires
Chest	<18 years*	• Blood sampling • Saliva sampling • Cardiac echography • Questionnaires
Others	<18 years	• Questionnaires
CNS: Tumor of the CNS, except diffuse pontine glioma or high grade glioma **CSI**: Craniospinal irradiation **H&N**: Other tumors located in the head & neck region, including upper thoracic aperture and cervical spine (e.g rhabdomyosarcomas and nasopharyngeal carcinoma) **Chest**: Tumors treated with mediastinal/chest/pulmonary irradiation, i.e. Hodgkin's lymphoma, Ewing sarcoma or other chest sarcomas, lung metastasis of Wilms and Ewing tumors *<22 years for France		

Biobank[6]

For patients treated for a tumor located in the CNS (focal irradiation and CSI), head, neck or chest, the research involves collection of 3 tubes of **blood** (12 mL in total) before radiotherapy, 3 tubes of blood (12 mL in total) immediately after or within 3 months after completion of radiotherapy, and 3 tubes of blood (12 mL in total) one year after completion of radiotherapy, for the assessment of biomarkers for cardiac and vascular damages or carcinogenesis (**3 timepoints**). For patients with thoracic EBRT or CSI, an additional tube of 4 mL is taken at each of the 3 timepoints for measurement of biomarkers of cardiac diseases (see Section 9.1). Whenever it is possible, the tubes are added to blood sampling performed anyway for routine care.

For patients with thoracic EBRT, the blood samples are taken as additional tubes collected at the time of blood sampling carried out as part of routine care. All other patients undergo blood sampling specifically for the research purposes.

The research also involves collection of **saliva** at the same timepoints (**3 timepoints**) as blood collection (4 mL in total at each timepoint).

This biobank aims to document the individual responses to the investigated biomarkers and the mechanisms underlying radiation induced second primary cancers and cardiac and vascular damages. A mechanistic understanding, together with biomarkers for individuals at increased risk, has the potential to increase the power of epidemiological studies regarding health effects of different radiotherapy modalities. The major advantage of saliva over blood is the non-invasive nature of collection that could lead to increased acceptability and participation of paediatric patients in research studies and the possibility of repeated sampling, which would facilitate molecular epidemiology research. More details are provided in Annex 8.

Neurovascular MRI sequences

Standard neurovascular MRI sequences: Patients with CNS tumours (focal irradiation or CSI) have routine MRI performed with injected intravenous contrast before radiotherapy for treatment planning

[6] Blood and saliva samples are taken for the research activities on cardiac and vascular damages. For the assessment of endocrine dysfunctions, blood hormone levels are measured on a routine basis, with no extra blood samples taken for the research.

and in their follow-up for assessment of tumour control. At follow-up timepoints (**3 timepoints**: years 1, 3 and 5 after completion of radiotherapy), neurovascular MRI sequences are added to the routinely performed follow-up MRI (see Section X). Compared to routine MRI exams, the neurovascular MRI sequences prolong the scanning time by 7 minutes per exam. These additional sequences are necessary to visualize the large and small vessels. No additional contrast medium than the contrast medium already routinely injected for tumour assessment is necessary.

At year 10 after completion of radiotherapy, the routine follow-up of the patient has usually finished. If the participant agrees, a non routine MRI injected with contrast may be performed for the purpose of assessing the neurovascular late damages. This non routine MRI (year 10) will require amendments to existing research protocol.

Baseline MRI assessment will be collected only if it was done as part of routine practice (optional timepoint).

Novel neurovascular MRI sequences: For Denmark (AUH), a novel neurovascular MRI sequence black blood is also added to the above described neurovascular sequences at the same timepoints. The research implies prolonging the scanning time by additional 7 minutes per scan at the same timepoints as described above (meaning 14 additionnal minutes in total).

Cardiac echography

For patients with thoracic irradiation, in addition to baseline (before radiotherapy) and follow-up (years 1, 5 and 10 after completion of radiotherapy) cardiac echography exams that are routinely carried out, the research involves an additional cardiac echography exam on the last day of radiotherapy or within the 3 months following the last fraction of radiotherapy which will be performed during a routine clinic visit (**1 timepoint)**.

Patients with CSI (CNS tumour) usually have no routine cardiac echography. For the research purposes, cardiac echography is then performed during a routine clinic visit at **5 timepoints** (before the first fraction of radiotherapy, on the last day of radiotherapy or within the 3 months following the last fraction of radiotherapy, at years 1, 5 and 10 after completion of radiotherapy).

Documentation by physician

For all patients, parent(s)/guardian(s) are asked about their educational level, profession, and occupational status at baseline (B0). Participants are asked about their academic achievement and professionnal and occupational status at all visits. For patients treated for a CNS, head or neck tumor, the physician or nurse also measures the participant's sitting body height, abdominal girth, hip size at each routine follow-up visits.

Questionnaires

The questionnaires consist of:

- HRQoL assessment (generic core scale – 23 items) and multidimensional fatigue (18 items), through the PedsQL™ Measurement Model, which is designed depending on the age of the child/adolescent. The questionnaires consist of reports from parent and/or child/ adolescent questionnaire (Table 6).
- Questions about academic achievement and potential key confounding factors (8 items) (Annex 5).

Questionnaires are completed over a maximum of a 10-year period after cancer diagnosis (and not after age 25), with **a maximum of 6 timepoints** (before EBRT, immediately after or within 3 months after completion of radiotherapy, years 1 and 3 after completion of radiotherapy, and years 5 and 10

after cancer diagnosis[7]). If the participant's 25th birthday occurs more than one year after a follow-up timepoint, the last questionnaire is completed at age 25. Parent-proxy participant questionnaires are conducted up to the participant's 18th birthday. The characteristics of the respondent (mother/father/guardian) are collected for statistical analysis purposes. The questionnaires are either completed during clinic visits (paper-based questionnaires), or at home (paper-based or electronic questionnaires).

Table 6. Description of the questionnaires completed by study participants and/or parent(s)/guardian(s)

Age of participant	Questionnaires	Total time (min)	Participant's report	Parents-proxy' report	Total questionnaires
1 - 12 months	• PedsQL™ Infant Scales™*	5	no	yes	1
13 - 23 months		5	no	yes	1
2 - 4 years	• PedsQL™ Generic Core Scales* • PedsQL™ Multidimensional Fatigue Scale* • Academic achievement	10	no	yes	3
5 - 7 years	• PedsQL™ Generic Core Scales* • PedsQL™ Multidimensional Fatigue Scale* • Academic achievement	10-15	yes**	yes	7
8 - 12 years			yes	yes	7
13 - 17 years			yes	yes	7
18 - 25 years	• PedsQL™ Generic Core Scales* • PedsQL™ Multidimensional Fatigue Scale* • Academic achievement	10	yes	no	3
*standard version **with help of an instructor such as a nurse or a clinical research assistant at the hospital, or parient(s)/guardian(s) if completed at home					

When the study participant reaches the age of majority, the subsequent questionnaires are only collected from the adult participant after signature of a new consent (see Section 7.2).

Duplication of PedsQL™ questionnaires for the present research and other studies should be strictly avoided. The investigator should check the subject's participation in a SIOPE trial or any other study assessing HRQoL. In particular, participants in SIOPE trials should not complete or receive PedsQL™ questionnaires at the following time points: before EBRT, one year after completion of radiotherapy, five years after cancer diagnosis, and age 18 years[8]. For these participants, only the questionnaire on academic achievement should be completed at these timepoints.

The timeline of all study participants' interventions are summarized in Table 7.

Table 7. Follow-up schedule for the study participants

	Tumor type (EBRT field)	B0	Follow up time (months after last day of EBRT)						
			M0	M12	M24	M36	M48	M60	M120
Informed consent	all	X							
Documentation by physician									
Parent(s)/guardian(s)' educational level	all	X							

[7] The questionnaires are completed at years 5 and 10 after cancer diagnosis (instead of years 5 and 10 after completion of radiotherapy) to allow having comparable timepoints with SIOPE trials.
[8] In SIOPE-BT trials, HRQoL is assessed by PedsQL™ questionnaires before cancer treatment, and then at years 2 and 5 after cancer diagnosis and at age 18. The timepoints Year 1 after completion of radiotherapy (HARMONIC) and Year 2 after diagnosis (SIOPE-BT trials) are considered to be equivalent.

Participants' educational level, profession, and occupational status	all	X		X	X	X	X	X	X
Anthropometric measurements *(sitting body height, abdominal girth, hip size)*	CNS, H&N	X		X	X	X	X	X	X
Imaging									
Cardiac echography	CNS-CSI	X	X	X				X	X
	Chest		X						
Neurovascular MRI sequences *(+ 7 min)*	CNS			X		X		X	X[2]
Neurovascular MRI black blood sequence *(+7 min)*[1]	CNS			X		X		X	X[2]
Biological sampling									
Blood sampling[3]	CNS H&N	X	X	X					
Blood bank (biomarkers of vascular diseases and carcinogenesis) – 1 EDTA tube, 1 serum separation tube, 1 CPT tube	CNS, H&N, Chest	X	X	X					
Volume per tube (mL)		*4*	*4*	*4*					
Total volume (mL)		*12*	*12*	*12*					
Blood bank (biomarkers of cardiac diseases: troponin, BNP, CPK) – 1 EDTA tube[8]	CNS-CSI Chest	X	X	X					
Volume per tube (mL)		*4*	*4*	*4*					
Total volume (mL)		*4*	*4*	*4*					
Total volume of blood in mL *(To be adjusted according to applicable national law regarding the maximal blood volume that can be collected in individuals)*	CNS-F	*12*	*12*	*12*					
	CNS-CSI	*16*	*16*	*16*					
	H&N	*12*	*12*	*12*					
	Chest	*16*	*16*	*16*					
Saliva bank (1 tube)[9]	CNS, H&N, Chest	X	X	X					
Volume per tube (mL)		*4*	*4*	*4*					
Total volume (mL)		*4*	*4*	*4*					
Questionnaires									
PedsQL™ (generic, fatigue)[4]	all	X	X	X		X		X[5,6]	X[5,6]
Academic achievement	all	X	X	X		X		X[5,6]	X[5,6]

[1] for Denmark only
[2] under further amendment
[3] For CNS and H&N tumor, patients undergo additionnal blood sampling for research purpose. Only patients with chest tumour, blood is taken as additional tubes collected at the time of blood sampling carried out as part of routine care
[4] if not collected otherwise in other studies, e.g. SIOPE trials
[5] if age ≤25 years
[6] the questionnaires are completed at years 5 and 10 after cancer diagnosis (instead of years 5 and 10 after completion of EBRT) to allow having comparable timepoints with SIOPE trials
[8] only performed if not routine exam (otherwise only lab results are collected)
[9] participants shall not eat 30 minutes before and shall wash the mouth or drink water

CNS: Tumor of the CNS, except diffuse pontine glioma or high grade glioma
CNS-F: Tumor of the CNS treated with focal irradiation;
CNS-CSI: Tumor of the CNS treated with craniospinal irradiation
H&N: Other tumors located in the head & neck region, including upper thoracic aperture and cervical spine (e.g rhabdomyosarcomas and nasopharyngeal carcinoma)
Chest: Tumors treated with mediastinal/chest/pulmonary irradiation, i.e. Hodgkin's lymphoma, Ewing sarcoma or other chest sarcomas, lung metastasis of Wilms and Ewing tumors

B0: between cancer diagnosis and inititiation of the first EBRT (the day of the first fraction at the latest)
M0: immediately after the last fraction of EBRT, or within 3 months after completion of EBRT
M12-M120: months 12 to 120 after the last fraction of EBRT +/- 6 months

7.3.2 Medical records

After prospective inclusion, data collection from existing medical records and hospital databases at the participating centres depends on the frequency of visits as part of routine care, i.e. every year or two years during the first 5 to 10 years after EBRT, and every year to every five years afterwards.

Data collection regarding the retrospective part is also made on a regular basis.

7.3.3 Linkage to Existing Mortality and Disease Registries and Healthcare Databases

Linkage to existing regional and/or national mortality or disease registries, and healthcare databases is done at the national level, following the regulation in place in each participating country. Unless written refusal, **the whole cohort (prospective and retrospective) is passively followed from childhood into adulthood** through the registries and databases listed in Table 8 based upon country-specific information avaibility as follows[9].

Table 8. Linkage of the cohort with mortality registries (vital status and causes of death), disease registries, and healthcare database

Passive follow-up	Country	Registry	Coverage of the registry
Vital status, causes of death, emigration status (if available)(1)	**Mortality registries**		
	Belgium	Belgian death registry	National All residents Age: 0 to 85+
	Denmark	-Danish Civil Registration System -Danish Register of Causes of Death https://econ.au.dk/the-national-centre-for-register-based-research/danish-registers/the-danish-civil-registration-system-cpr/	National since 1973 All residents Age: 0 to 85+
	France	-Répertoire national d'identification des personnes physiques (RNIPP) -Centre d'épidémiologie sur les causes médicales de Décès (CépiDC)	National since 1968 All residents Age: 0 to 85+
Long-term endpoints, including endocrine and vascular diseases, and second and subsequent primary cancers, occurring mainly after 10 years of follow-up	**Disease registries**		
	Belgium	Belgian Cancer Registry http://kankerregister.org	National since 2004 All residents Age: 0 to 85+
	Denmark	Danish Childhood Cancer Registry https://www.danishhealthdata.com/find-health-data/Dansk-Boernecancer-Register?disallowCookies=1	National since 1985 All residents 1985+, age: <15 2018+, age <18
		Danish Register of Congenital Heart Disease	National since 1963/77 All patients treated at Danish hospitals Age: 0 to 25
		Danish Stroke Registry (acute stroke, transient ischemic attack) https://www.danishhealthdata.com/find-health-data/Dansk-Apopleksi-Register?disallowCookies=1	National since 2003/13 All patients treated at Danish hospitals Age: ≥18 to 85+
		Danish Heart Registry (ischaemic cardiac and/or valvular diseases treated with invasive cardiac procedure) https://www.danishhealthdata.com/find-health-data/Dansk-Hjerteregister	National since 2003/06 All patients treated at Danish hospitals Age: >15 to 85+
	France	National Childhood cancer registry (RNCE)	National since 2000 All residents 2000+, age: <15 2011+, age <18
		Regional cancer registries (FRANCIM)	Regional since 1975 (~20% of residents)
	Germany	Regional Cancer registries	National since 1999 All residents 1980+, age: 0 to 15

[9] Linkage to healthcare databases also allows retrieving exposures to other medical procedures using ionizing radiation (type and number of procedures, date of receipt), such as CT scans, received for cancer diagnosis and cancer sequalae surveillance and management, which may have a substantial contribution to the irradiation of out-of-field organs and subsequent risks of radiation-related late effects (see Sections 1.3.2 and 12).

			2009+, age: 0 to 85+
		German Childhood Cancer Registry www.kinderkrebsregister.de	National since 1980 All residents 1980+, age: 0 to 15 2009+, age: 0 to 18
	Healthcare databases		
	Belgium	Inter Mutualistic Agency (reimbursement claims: in-/out-patient + ambulatory, public and private)	National since 2002 All residents Age: 0 to 85+
	Denmark	Danish National Patient Registry (reimbursement claims: somatic and psychiatric in-/out-patient, public and private)	National since 1977/2003 All residents Age: 0 to 85+
	France	National inter-health insurance scheme information system (SNIIRAM)[(2)] (reimbursement claims: in-/out-patient + ambulatory, public and private)	National since 2006/11 All residents Age: 0 to 85+
[(1)]Emigration status available in Denmark only [(2)]Data collection from Sniiram is subject to compliance with applicable security referential as required by national law			

7.4 Individual Dosimetry

At each centre, DICOM format files for radiation treatment plan (DICOM-RT plan, structure, dose sets) are extracted from the local treatment planning systems. These data, combined with computational phantoms and analytical models, are used for whole-body and non-targeted organ dose reconstruction, so as to complement and extend the calculations performed by the treatment planning system for all study participants. In the framework of HARMONIC, two analytical model computations will be employed for the whole-body dose calculation, one model devoted to photon beams and the other for PBT. The results of the analytical models will be validated in selected cases using Monte Carlo simulations. For photon EBRT, the dose verification system PRIMO (based on PENELOPE) will be used. For proton EBRT, the Monte Carlo treatment planning system Raystation will be employed. The accuracy of Raystation for computing out-of-field doses has been already evaluated within HARMONIC by means of experimental measurements and comparison with the general-purpose Monte Carlo code Geant4 (article submitted for publication in a peer-reviwed journal). Further experimental evaluation of the analytical and Monte Carlo codes that will be used is foreseen by means of irradiation of anthropomorphic phantoms with photon and proton beams. The experiments and simulations will also consider the absorbed dose distributions obtained from imaging procedures. Imaging doses will be computed using the general purpose Monte Carlo code PENELOPE. After experimental validation of every imaging system on anthropomorphic phantoms, individualized calculation will be performed using patient specific models [117] based on their own DICOM CT image (image used by the TPS). More details are provided in Annex 7.

7.5 Visit of Early Study Withdrawal

Not applicable

7.6 Study Participant's Withdrawal from Research

The subject's participation in the research is voluntary and the participant may refuse to participate or withdraw from the research, at any time, without penalty or loss of benefits to which the participant may be otherwise entitled. For evaluation and reporting purposes, participants may be asked for their reasons for withdrawal.

The subject's participation may also be terminated at any time at the investigator's discretion. Whenever an investigator terminates a subject's participation in research, the investigator must explain to the participant the reasons for the early termination.

In case of withdrawal, no intervention for the research shall be carried out after the date of withdrawal. Data already obtained before withdrawal can still be processed and analyzed unless written refusal.

Unless written refusal, the participants who withdraws consent will be followed "passively" (this follow-up will not require the subjects' participation) through linkage with national/regional mortality and disease regsitries, administrative and healthcare databases and/or through medical records. Indeed, for scientific purposes, it is necessary to follow all the eligible population and avoid potential biaises due to withdrawal or dropouts due to health and/or social status or outcomes. The data already obtained before withdrawal can still be processed and analyzed on the basis of the previoulsy signed and informed consent.

Any participant who does finally not receive EBRT, or whom DICOM-RT data cannot be retrieved, will be excluded from the study (prospective inclusion part). No other exclusion criteria beside withdrawals and dropouts of participants from the research apply.

The research will not affect treatment and no individual benefits are expected.

7.7 End of Research

7.7.1 Definition of End of Research

The end of the research corresponds to a 10-year period following the last day of the 10-year period of inclusion (31 May 2040 as anticipated date). Pending further protocol's amendement, this period may be extended.

7.7.2 Description of Rules applying to Definitive or Temporary Termination of All or Part of the Research

In accordance with applicable national laws of the participating countries, if the inclusions have not commenced within the regulatory period following the receipt of the favorable opinion of the Research Ethics Committees, the agreement of the Research Ethics Committees will be deemed to lapse and the research will have to be resubmitted to the applicabe Research Ethics Committees in order to be extended. This request for extension should be accompanied by a letter justifying the delay in relation to the original research timetable. During the research, if the rate and number of inclusions appear insufficient, the sponsor[10] may decide to stop it if no other solution can be envisaged.

The sponsor or the competent authorities may interrupt the research for any other justified reason (e.g. major deviations from the protocol which do not guarantee the safety of the participants, the quality of the data and the results of the research).

8 ADMINISTERED PRODUCTS

Not applicable

9 STUDIED PARAMETERS

9.1 Definition of the studied parameters and measurement techniques

Endocrine dysfunctions

- Blood hormone levels (lab test results)[11]
    - insulin-like growth factor-1 (IGF-1)
    - anterior pituitary hormones (GH, ACTH, TSH, LH, FSH)
    - thyroid hormones (fT3, fT4)

[10] Definition of „sponsor" under the consortium agreement
[11] as measured routinely

    - sexual hormones (estrogen, testosterone, progesterone)
- Anthropometric mesaurements
    - Body mass index (weight in kg / standing body height in meter^{10})
    - Sitting body height; sitting body height-to-standing body height ratio
    - Abdominal girth
    - Hip size
- Pubertal development
- Hormone replacement therapy
- Endocrinopathies (e.g. hypothyroidism, growth hormone deficiency), coded according to the applicable version or edition of the Common Terminology Criteria for Adverse Events (CTCAE) or the International Classification of Diseases (ICD).

Cardiovascular diseases damages

- Early changes in imaging markers of cardiovascular damages (cardiac echography)
    - ejection and shortness fraction
    - others
- Early changes in blood markers of cardiovascular damages
    - Troponin
    - BNP
    - CPK
- Early changes in blood/saliva markers of other vascular damages – see Annex 8
    - Signal quality of protein activity assessed by RPPA
    - inflammatory markers, e.g. PTX3, CRP, NF-kB, IL-1 and IL10
    - markers of oxidative stress, e.g. 8-oxo-dG, SOD2, DNA repair enzymes and other potential circulating biomarkers
- Cardiovascular diseases (e.g. heart failure)

Neurovascular damages

- Changes in imaging markers of neurovascular injury on MRI imaging. The following sequences are used: T1 (longitudinal relaxation time weighted) with and without contrast, T2 (transverse relaxation time weighted) or Flair (fluid attenuation inverse recovery), SWI (susceptibility weighted images) or T2-STAR (transverse relaxation time weighted in gradient echo sequences), MRAngio (MRI angiography) or TOF (time of flight), DWI (diffusion-weighted imaging), and, for Danish patients only, “Black Blood” novel neurovascular sequence for detection of inflammation in the vessel wall, for evaluation of:
    - Small vessel disease burden: number of microbleeds, cavernoma, white matter changes evaluated by a modified Klarenbeek score;
- Large vessel disease: extent of stenosis and/or inflammation in the large intracranial vessels.Early changes in blood/saliva markers of vascular damages – see Annex 8
    - Signal quality of protein activity assessed by RPPA
    - inflammatory markers, e.g. PTX3, CRP, NF-kB, IL-1 and IL10
    - markers of oxidative stress, e.g. 8-oxo-dG, SOD2, DNA repair enzymes and other potential circulating biomarkers
- Clinical neurovascular damages (e.g. stroke, transient ischemic attaque, cerebral hemorrhage)

Second primary cancers

- Blood/saliva markers of carcinogenesis – see Annex 8:
    - leukocyte telomere length (LTL)
    - mitochondrial DNA copy number (mtDNAcn)
    - circulating microRNA (miRNA), e.g. miR-18a, miR-21, miR-155, miR-221, and miR-375
- Diagnoses of second or subsequent primary cancers, coded according to the applicable edition of the ICD for Oncology

- malignant or in situ tumours (any localization or histology)
- all CNS tumours (any histology or behaviour)

HRQoL and fatigue

The Pediatric Quality of Life Inventory (PedsQL™) Measurement Model (Annex 4) is used to measure HRQoL and multidimensional fatigue:

- PedsQL™ core scale (physical, emotional, social, and school functioning – 23 items)
- PedsQL™ multidimensional fatigue scale (general, sleep/rest, and cognitive fatigue – 18 items)

The PedsQL™ is a validated modular approach to measuring HRQoL in healthy children, adolescents and young adults, and those with acute and chronic health conditions. It is designed depending on the age of the respondant.

Academic achievement

A short questionnaire (8 items), specifically designed for the present research (Annex 5), is used to measure academic achievement:

- Achieved grade
- Repeating grade(s)
- Use of special education services

Other late morbidity (if not mentioned above)

- CTCAE (applicable version)
- ICD (applicable edition)

All-cause and cause-specific mortality

- ICD (applicable edition)

9.2 Methods and timeline for measurement, collection, and analysis of the studied parameters

For scientific, ethical and technical reasons, the studied parameters vary according to characteristics of the study participants, i.e. treated tumors / EBRT fields, age at first EBRT, and possibility of re-irradiation (Table 9). Blood sampling is carried out in agreement with the legal requirements in effect in each country regarding the maximal blood volume that be collected in individuals (see Section 10).

Table 9. Study participants' characteristics for measurements, collection, and analysis of the studied parameters

	Age at first EBRT (in years)	1st EBRT	Tumor type / EBRT fields	Other selection criteria
Endocrine dysfunctions • Blood hormone levels • Anthropometric measurements • Pubertal development • Hormone replacement therapy • Endocrinopathies	0 to < 18	X	CNS H&N	*None*
			All patients	
Cardiovascular damages • Imaging markers of cardiovascular damages • Blood markers of cardiovascular damages • Blood/saliva markers of vascular damages	0 to < 18*	X	CNS-CSI Chest	Informed consent; Legal requirements regarding the maximal blood volume that be collected

• Cardiovascular disease			*All patients*	*None*
Neurovascular damages • Imaging markers of neurovascular damages	0 to < 18	X	CNS	Informed consent
• Blood/saliva markers of vascular damages		X	CNS H&N	Informed consent; Legal requirements regarding the maximal blood volume that be collected
• Neurovascular disease			*All patients*	*None*
Second primary cancers • Blood/saliva markers of carcinogenesis	0 to < 18*	X	CNS H&N Chest	Informed consent; Legal requirments regarding the maximal blood volume that be collected
• Second or subsequent primary cancers			*All patients*	*None*
HRQoL and academic achievement • PedsQL™ core scale	0 to < 18*		*All patients*	Informed consent; Patients and/or parent(s)/guardian(s) willing and able to comply with fulfilling questionnaires; HRQoL before and one year after EBRT not already collected otherwise (e.g. in the framework of a clinical trial)
• PedsQL™ multidimensional fatigue scale	2 to < 18*			
Other late morbidity • CTCAE • ICD	0 to < 18*		*All patients*	*None*
Academic achievement	2 to < 18*		*All patients*	*None*
All-cause and cause-specific mortality • ICD	0 to < 18*		*All patients*	*None*
*under 22 years for France for patients with mediastinal/chest/pulmonary irradiation **CNS**: Tumor of the CNS **CNS-CSI**: Tumor of the CNS treated with craniospinal irradiation **H&N**: Other tumors located in the head & neck region, including upper thoracic aperture and cervical spine (e.g rhabdomyosarcomas and nasopharyngeal carcinoma) **Chest**: Tumors treated with mediastinal/chest/pulmonary irradiation, i.e. Hodgkin's lymphoma, Ewing sarcoma or other chest sarcomas, lung metastasis of Wilms and Ewing tumors				

The timeline for measurement, collection, and analysis is also specific to the studied parameters (Table 10).

Table 10. Timetable for measurements and collection of the studied parameters, combining information collected within the framework of the research or on a routine basis

		Follow up time (months after last day of RT)							
		(active / passive follow-up)							*(passive)*
	B0	M0	M12	M24	M36	M48	M60	M120	>M120-M240
Endocrine dysfunctions									
• Blood hormone levels	X	X	X	X	X	X	X	X	
• Anthropometric measurements	X	X	X	X	X	X	X	X	
• Pubertal development	X	X	X	X	X	X	X	X	
• Hormone replacement therapy	X	X	X	X	X	X	X	X	X
• Endocrinopathies	X	X	X	X	X	X	X	X	X
Cardiovascular damages									
• Imaging markers of cardiovascular damages (cardiac echography)	X	X	X				X	X	
• Blood markers of cardiovascular damages	X	X	X						
• Blood/saliva markers of other vascular damages	X	X	X						
• Cardiovascular diseases	X	X	X	X	X	X	X	X	X
Neurovascular damages									
• Imaging markers of neurovascular damages (MRI imaging)	X		X		X		X	X[1]	

• Blood/saliva markers of vascular damages	X	X	X						
• Neurovascular diseases	X	X	X	X	X	X	X	X	X
Second primary cancers									
• Blood/saliva markers of carcinogenesis	X	X	X						
• Second or subsequent primary cancers			X	X	X	X	X	X	X
HRQoL and academic achievement									
• PedsQL™ core scale	X	X	X		X		X^2	X^2	
• PedsQL™ multidimensional fatigue scale	X	X	X		X		X^2	X^2	
• Academic achievement	X	X	X		X		X^2	X^2	
Other late morbidity									
• CTCAE	X	X	X	X	X	X	X	X	X
• ICD	X	X	X	X	X	X	X	X	X
All-cause and cause-specific mortality									
• ICD	X	X	X	X	X	X	X	X	X

B0: between cancer diagnosis and inititiation of the first EBRT (the day of the first fraction at the latest)
M0: immediately after the last fraction of EBRT, or within 3 months after completion of EBRT
M12-M120: month 12 to 120 after the last fraction of EBRT +/- 6 months
[1] under further amendment of the protocol
[2] without exceeding the age of 25 years

10 BIOLOGICAL SAMPLES

Collection of blood and saliva allows biosamples to be available in biobanks for future studies aiming to identify/validate new biomarkers of late health effects of diseases.

10.1 Sample Collection

Blood and saliva samples are collected by an authorized (nurse) person at the hospitals. Blood sampling is carried out in agreement with the legal requirements in effect in all countries regarding the maximal blood volume that be collected in individuals. Blood collected will have to be adjusted according to weight, clinical condition and blood taken for other research or routine care purposes.

Blood is collected in patients with CNS, head and neck, and chest tumors at 3 routine visits: 1) before start of radiotherapy (B0), 2) immediately after completed exposure or anytime up to 3 months after radiotherapy (M0) and 3) one year after completion of radiotherapy (M12). At each timepoint, 12 ml are collected into 3 tubes: one test tube containing EDTA K2 (4 ml), one plastic clot activator serum separation tube (4 ml) and in a BD Vacutainer® CPT ™ tube for isolation of lymphocytes (4 ml). For patients with thoracic or craniospinal irradiation, an additional EDTA tube of 4 mL is collected at each timepoint for measurement of cardiac diseases markers (troponin, BNP, CPK).

Saliva is collected at 3 routine visits: 1) before start of radiotherapy (B0), 2) immediately after completed exposure or anytime up to 3 months after radiotherapy (M0) and 3) one year after completion of radiotherapy (M12). At each timepoint described above, 4 ml saliva are collected in a sterile standard (any brand) plastic tube (5 or 10 ml) without any additive. Patients must not eat 30 minutes before giving a saliva sample, and must wash the mouth or drink water prior to saliva sampling.

10.2 Coding and labelling

Coding and labelling are done at the hospital after preparation of samples by a nurse or other dedicated medical staff. All collected samples, in any form, are identified through a unique participant identification number (14 digits), without indication of the family name or first name or any other personal data that could allow direct identification of paediatric participant. The hospital name shall not be affixed to tubes.

10.3 Sample Pre-Analytical Processing

Each blood tube are centrifuged within two hours post-collection to obtain plasma, serum (from coagulated blood) and lymphocytes:

- The EDTA tubes are centrifuged at about 300xg (2500 RPM in a standard centrifuge) for 10 minutes at + 4 °C. The supernatant (plasma) and the cells (bottom pellet fraction) will be frozen in separated tubes in a -20°C freezer. The samples should be kept in -80°C if available.
- The plastic tubes without anticoagulants are kept for 1 hour at ambient temperature in order to facilitate the coagulation. After coagulation, the tubes are centrifuged at 500 x g for 10 minutes. After centrifugation, the supernatant (serum) are kept in a -20°C freezer and, if possible, the blood clot should be kept in the -20°C freezer for future use (DNA, miRNA and RNA can be isolated). The samples should be kept in -80°C if available.
- The BD Vacutainer® CPT™ tubes are centrifuged to separate lymphocytes from plasma and red blood cells. The lymphocytes are prepared according to a separate protocol and then kept at -20°C. The samples should be kept in -80°C if available.

The saliva are collected in sterile plastic tubes without any additive and kept in a -20°C freezer within 5-10 minutes. Saliva samples should be stored at least -18°C.

10.4 Transport

In accordance with each national applicable laws and requirements, blood and saliva samples are sent from hospitals to Sweden SU, in boxes containing dried ice to keep the samples (blood, serum, plasma) frozen during the transport. Transport is done by an authorized delivery company to the SU, Sweden and from there the samples are distributed to the other partners in France and Italy for analyses. The preparation of samples for shipment and the receipt of samples is strictly done by the staff in charge of the project.

10.5 Storage and/or Destruction

The blood samples (serum, plasma and cells) are kept at -20°C for short-term storage (6 months) and -80 °C for long-term storage. The saliva samples are kept at -20°C as soon as the samples are collected. The biobank (blood and saliva samples) is centralized at Sweden SU under the responsibility of Dr. Siamak Haghdoost.

If the biological samples (blood and saliva) collected is not used in its entirety at the end of the research, it will be sent back to the original centres upon request. Otherwise, it will be stored at Stockholm University under the responsibility of Dr. Siamak Haghdoost, for a maximal period of 10 years after the end of the project, for later use in other research on late effects of cancer treatments or individual susceptibility to late diseases after cancer with due regard to confidentiality.. The room, E205, is located at Stockholm University, Svante Arrheniusvag 20C, Stockholm. The locked and alarmed room contains several freezers which are monitored 24/24h.

10.6 Quality Assurance of Collection

As quality control is a fundamental requisite in the management of biological samples as well as in the biomarkers studies, the research laboratories will adhere to all standard operating procedures (SOP). SOP will be applied in the processing, storage and analyses of the biological samples. For instance, collected samples will be stored in multiple aliquots in small vials in at least two different physical locations to avoid the likelihood of loss of the sample as a result of accidental thawing due to freezer failure or electronic blackout. The samples will be stored in freezers that have a 24-hour monitoring through a computerized alarm system.

11 VIGILANCE

Any investigator who is aware of, or becomes aware of, a health safety risk, which originates from any of the biological materials transferred, shall inform the other involved investigators without delay and provide them with all the information in its possession or at its disposal concerning risks of this kind.

12 DATA COLLECTION AND MANAGEMENT

Personal data are collected and processed strictly for the purposes of the research described in the present protocol. Data processing includes the management of personal data of the participants to the research with the aim of data collection, entry, quality and coherence control, and statistical analyses. In HARMONIC, data collection and management follows the general rules of the Data Mangament Plan attached in Annex 9. The Data Management Board includes representative data managers of each institution as described in the Data Mangament Plan.

12.1 Description of the Collected Data

Only personal data that are strictly necessary and relevant to the objectives of the research are collected as showed in Table 11.

As a general rational, the data collected will allow the assessment of late outcomes of EBRT, which typically occur years to decades after treatment, through active and passive procedures, and the assessment of intermediate biological and imaging markers (at the end of EBRT, or 1 to 3 years after treatment) which can correlate to late outcomes.

Table 11. List of collected data for the research purposes

Collected data	Description	Rationale
Identification (non-pseudonymised)*	Full name Date of birth (dd/mm/yyyy) Place of birth (country and city) National civil identification / health insurance number Referring center	Identifiers necessary for passive, long-term follow-up through external registries/databases
Identification (pseudonymised)	Informed consent, date of informed consent Date and center of inclusion	Identification, eligibility criteria
	Participation in a clinical trial / registry	Eligibility criteria for specific procedures (e.g. questionnaires) + cancer treatment information
Contact information (non-pseudonymised)*	Parents'/legal representatives' email address Major patients' email address Parents'/legal representatives' postal address Major patients' postal address	Mean of contact for follow-up by questionnaire HRQoL and academic achievement
Contact information (pseudonymised)	Date of last contact/reasons for lost-of-follow-up Vital status, date and causes of death	Follow-up
	Emigration status, date of emigration	Data necessary for follow-up purposes (follow-up ending at the emigration date)
Demographics and socioeconomics	Sex Year and month of birth	Potential confounders in statistical analyses
	Usual and current place of residence (zip code, city, state and country) Health insurance coverage/scheme	Socioeconomic status / social inequality necessary for social impact assessment (outcomes) and potential confounders in statistical analysed (health insurance, zip code: area deprivation indexes) Distance to the cancer treatment center (follow-up + assessment of indication bias when comparing different EBRT techniques)
Health	Primary cancer characteristics Cancer and non-cancer treatment data (surgery, radiotherapy, chemotherapy,	Cancer treatment characteristics, potential confounders or effect modifiers of radiotherapy-related long-term outcomes

	other cancer treatments, anaesthesia/sedation, hormone replacement therapy, corticosteroids, antiplatelet aggregation medication or antico-agulants, supportive therapy) Contraception	
	Patient's use of psychological services Disability pension attendance Use of special services in school such as an individualized education program, FM hearing system	HRQol and academic achievement end-points
	General health status Personal and family cancer and vascular dis-ease history Genetic syndromes General health status Comorbidities Renal function, metabolic profiles (routine lab tests)	Potential confounders in analyses of risks of radiotherapy-related long-term out-comes
	Height, weight, abdominal girth, hip size Pubertal developpement Serum hormone levels (routine lab tests)	Endocrine endpoints Potential confounders in analyses of risks of radiotherapy-related long-term non-endocrine outcomes
	Cardiac echography reports	Cardiovascular endpoints
	Tumor control, relapse/progression Endocrine dysfunctions Cardiovascular diseases Neurovascular diseases Second primary cancers Other cancer treatment toxicities Health-Related Quality Of Life (physical, emo-tional, social and school functionings) and fatigue from patient and parent/legal repre-sentative	Primary and secondary endpoints (measurments at baseline necessary to adjust the statistical analyses for other treatment toxicities and tumor- or other disease-related sequelae)
Imaging	DICOM-RT data (RT plan, RT structure, RT dose) RT machine (models, commissioning data) Diagnosis/treatment plan/repositioning CT/MRI images Imaging protocols (non individual data): system (model, maker, version) and acquisition pa-rameters Number of images performed during treatment	Data necessary for dose reconstruction (in-field and out-of-field organs and tis-sues)
	Neurovascular MRI images	Data necessary for assessment of neuro-vascular endpoints
Biological samples	Blood sample Saliva sample	Data necessary for assessment of sec-ondary endpoints (biomarkers of vascular diseases, or carcinogenesis / second cancers)
Professional life	Parents' educational level, profession, occupa-tional status	Adjustment factors for analyses of primary endpoints (HRQoL and academic achievement, vascular diseases, others)
	Patient's educational status/achievement, use of special education services, profession, oc-cupational status	HRQol and academic achievement end-points
Personnal life	Physical activity and sedentariness Time spent watching TV/computer/tablets	Adjustment factors for analyses of primary endpoints (HRQol and academic achievement, others)
	Smoking history, alcohol and drug consumption	Adjustment factors for analyses of primary endpoints (vascular diseases, second cancers, others)
Mortality	Vital status, date and causes of death	Data necessary for follow-up purposes + assessment of secondary endpoints
Disease registries	Diagnoses, severity (grade or stage), date of diagnoses, comorbidities	Data necessary for long-term follow-up purposes (after the end of participants' follow-up at the inclusion center), i.e. as-sessment of primary and secondary end-points
Healthcare data-bases (reimburse-ment claims)	Clinical diagnoses, type of procedures, date of diagnoses and procedures	

		Data necessary for the assessment of exposures to medical procedures using ionizing radiation, in addition to radiotherapy and diagnostic imaging for EBRT planning and repositioning (data collected at the participating centers), mainly CT scans for cancer diagnostic and follow-up and management of subsequent morbidity, which may contribute substantially to out-of-field doses and bias results for radiotherapy-related risks of health events (e.g. CT scans related to presumed risks of late toxicities of radiotherapy)
*This information is strictly kept at the inclusion center and authorized entity (i.e. Inserm for French patients only) level; no transfer of non-pseudonymised data to the centralized database or other investigators is allowed.		

Coding of causes of death is performed according to the applicable edition of the ICD. Tumor characteristics are classified with the ICD for Oncology. Other health events are classified according to the applicable version of the CTCAE.

Non-pseudonymised identifying data (full name, exact date and place of birth, national unique identification number, referring center, and contact information) are only collected for the purposes of linkage with national/regional mortality and disease registries and national healthcare database to allow a passive long-term follow-up. As it is described in Section X, cancer treatment related-morbidity mostly occurs years to decades after treatment, while pediatric cancer survivors are not followed at the treatment center anymore. As a consequence, it is crucial and indispensable to continue to follow the study participants through these registries and databases, which requires the collection of identifying data. Only identifying data necessary for the linkage are collected. These data are strictly kept in a secured manner at the investigating centers and authorized entities (i.e. Inserm unit 1018 research group "Radiation Epidemiology", for patients recruited from the French centers only), together with a unique participant study ID (see Section X). These data are transferred by no means to the centralized database (see Section X), other investigating centers, or third parties. The national civil identification / health insurance number corresponds to the *Identificatienummer van de sociale zekerheid* (*INSZ)* in Belgium, *Numéro d'Inscription au Répertoire (NIR)* in France, and *Det Centrale Personregister* / Civil Personal Registration (CPR) number in Denmark. No such identification number is collected in Germany. Data collected and processed from linkage are relevant and limited to the purpose of the research project in accordance with the `data minimisation´ principle.

For practical reasons, **linkage for passive follow-up** will be done every two years starting in 2023 by the investigating center, or authorized entity at the national level (i.e. Inserm for French patients only). Data access from mortality and disease registries and healthcare databases are either obtained by direct matching with national civil identification / health insurance number, or by probabilistic matching based on personal identifiers, depending on the availability of the national civil identification / health insurance number (Table 12). The national civil identification / health insurance number are obtained in medical records when retrospective inclusion or directly from the participant at the time of approval to participate. When the national civil identification / health insurance number is not available or retrieved, the probabilistic matching based-method shall be used. Only pseudonymised data, after removal of the identifying information used for cohort linkage, will be transferred to the centralized database.

Table 12. Description of the personal information required for linkage and procedures of linkage with external registries and databases for passive follow-up

Country	Registry	Personal information required for linkage	Procedures of linkage
Mortality registries (vital status and causes of death)			

Belgium	Belgian death registry	tbd	tbd
Denmark	-Danish Civil Registration System -Danish Register of Causes of Death	CPR	Exact matching
France	-Répertoire national d'identification des personnes physiques (RNIPP) -Centre d'épidémiologie sur les causes médicales de Décès (CépiDC)	Full name, date and place (city, country) of birth, sex	Probabilistic matching
Disease registries (long-term endpoints)			
Belgium	Belgian Cancer Registry	INSZ	Exact matching
Denmark	Danish Childhood Cancer Registry	tbd	tbd
	Danish Register of Congenital Heart Disease	CPR	Exact matching
	Danish Stroke Registry	CPR	Exact matching
	Danish Heart Registry	CPR	Exact matching
France	National Childhood cancer registry	Full name, date of birth, sex*	Probabilistic matching
	Regional cancer registries (FRANCIM)	Full name, date of birth, sex*	Probabilistic matching
Germany	Regional Cancer registries	tbd	tbd
	German Childhood Cancer Registry www.kinderkrebsregister.de	tbd	tbd
Healthcare databases (long-term endpoints)			
Belgium	Inter Mutualistic Agency	tbd	tbd
Denmark	Danish National Patient Registry	CPR	Exact matching
France	National inter-health insurance scheme information system (SNIIRAM)	NIR (when available, for study participants prospectively included only) Otherwise, Full name, date and place (city, country) of birth, sex	Exact / probabilistic matching
*additional information, e.g. cancer treatment center, place of usual residence, could be used to improve the matching procedure performance Tbd: to be defined			

12.2 Definition of Source Data

The source data is all the information contained in original documents, or in authenticated copies of these documents, which relate to clinical examinations, observations or other activities carried out as part of a research involving the person which are necessary for the reconstruction and evaluation of research. The documents in which the source data is saved are called the source documents, whatever the medium used (paper, electronic ...).

The different sources of information used for the research are described in Table 13.

Table 13. Description of the source documents used for data collection

Source documents	Source data	Support
PedsQL (child and proxy reports)	HRQoL and academic achievement questionnaires	Paper or electronic
Mortality registries	Vital status, date and causes of death, emigration	Electronic
Healthcare databases	Reimbursement claims	Electronic
Disease mortality	Diagnoses of cancers and vascular diseases	Electronic

Picture archiving and communication system of each participating center	CT, MRI images	Electronic (DICOM)
	DICOM-RT data	Electronic (DICOM)
Document form for approval to participate	National civil identification / health insurance number E-mail/postal address	paper
Lab test reports	Endocrine hormone levels, renal function, metabolic profiles, cardiac disease markers	Paper or electronic
Radiology reports	Physician's interpretation of cardiac echography and neurovascular MRI	Paper or electronic
Medical records*	Vital status, date of death, last contact information baseline and follow-up tumour characteristics and outcomes, cancer and non-cancer treatments, participation in clinical trials, contraception, patient's use of psychological services, disability pension attendance, use of special services in school, general health status, personal and family health history, genetic syndromes, general health status, comorbidities routine lab test results and interpretation height, weight, abdominal girth, hip size, pubertal developpement endocrine dysfunctions, cardiovascular diseases, Neurovascular diseases, Second primary cancers, Other cancer treatment toxicities Professional life Personnal life	Paper or electronic
Administrative databases of the participating center	National civil identification / health insurance number Date and place of birth Current and usual place of residence Vital status, date of death, last contact information	Electronic
e-CRF	E-mail/postal address Place of birth height, weight, abdominal girth, hip size, pubertal developpement HRQoL and academic achievement anthropometric measures (abdominal girth, hip size, sitting body height), pubertal development family cancer history, medications Professional life Personnal life	Electronic
*Kiproreg local registry at UK Essen; Danish Childhood Cancer Registry at AUH		

12.3 Circuit of Data

An overview of the circuit of data is provided in Figure 4.

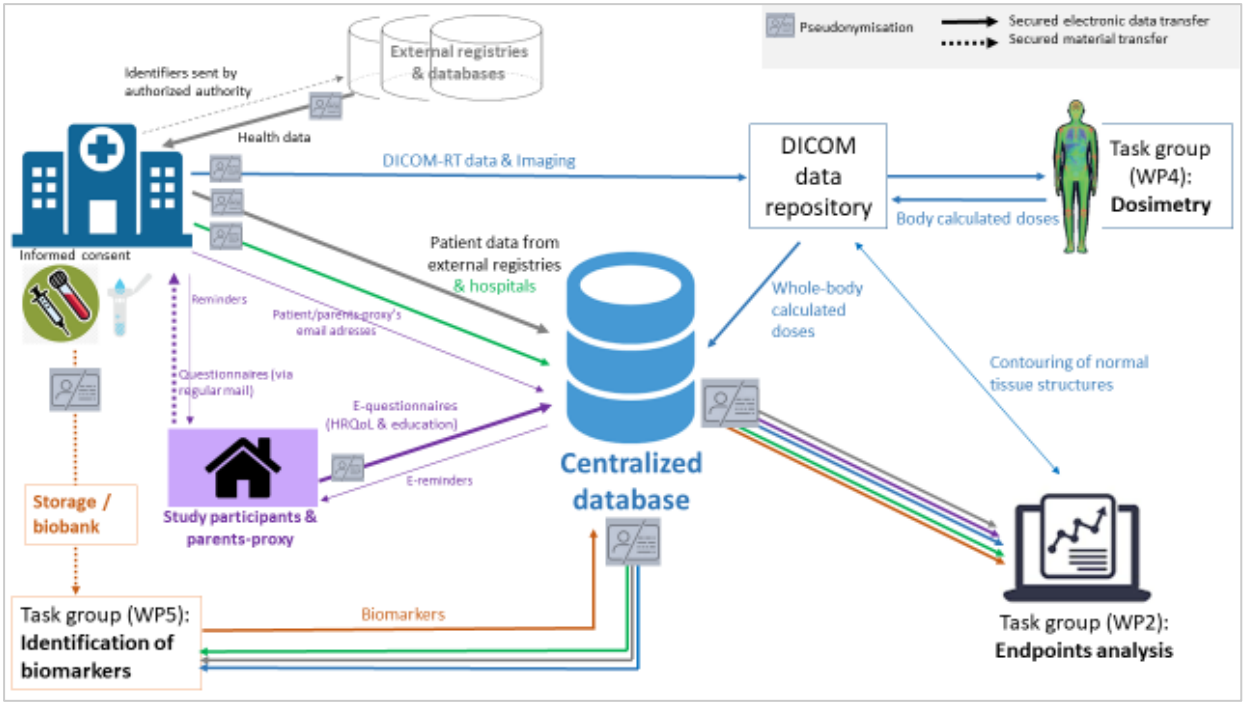


Figure 4. Overview of the circuit of data

Informed consent signed

A copy of the informed consent/assent forms signed by the participants and/or the legal representatives is kept by the investigators at each center. Another copy to the attention of the sponsor must be kept in a secure manner during the legal period for document conservation. Each center has physical security measures for premises. A third copy is given to the participants and/or the legal representatives. Data entry (date of consent, date and center of inclusion) is carried out at each participating center in an electronic CRF.

Questionnaires

At baseline, the questionnaires are completed at the inclusion clinic visit. During follow-up, paper-based questionnaires are completed during clinic visits on the last day of radiotherapy (or within the 3 months following the last fraction of radiotherapy), and at any other timepoint of research interventions (blood/saliva sampling, MRI exams, or cardiac echography). In any other case or when the questionnaires have not been completed during the clinic visit for any reason, paper-based questionnaires or an invitation to complete the questionnaires online on a secured platform (Redcap – see Annex 9) are sent at home by regular mail and/or by email to the parents who provided their email addresses to the investigator, after checking the study participant's vital status (through medical records, or linkage of the cohort to national mortality registries). Paper-based and electronic questionnaires contain no personal identifiers.

The completed paper-based questionnaires are sent by the investigator (if completed during a clinic visit) or directly by the participant (if completed at home) to Inserm unit 1018 research group "Radiation Epidemiology" for data entry. The paper-based questionnaires which are received by Inserm are strictly anonymized with the participant study ID. Inserm shall keep a copy of the anonymized questionnaires until the end of the research to allow possible rectification of entry errors.

E-mail invitations and reminders for follow-up questionnaires are sent automatically through the secured platform, with access rights to the e-mail addresses strictly restricted to the investigator. A reasonable number of reminders for completion is sent to participants.

Data are stored in the centralized core database.

Blood and saliva sample

Blood and saliva samples are collected and stored at each center before shipment to Sweden SU. From there, the samples will be distributed to the other WP5 partners in France (GR) and Italy (IFC-CNR) for analyses. If the biological samples collected are not used in its entirety at the end of the research, it will be sent back to the original centers upon request. Otherwise, it will be stored at Sweden SU under the responsibility of Dr. Siamak Haghdoost for later use in other research on late effects of cancer treatments or individual susceptibility to late diseases after cancer with due regard to confidentiality. Pseudonymized results of biological analyses are stored in the centralized database .

Hospital-based data from individual medical and administrative records
Data entry is carried out at each participating center in an electronic CRF.
Abdominal girth and hip size are obtained during medical examination. All other clinical and medical data are obtained from individual medical records. These data are pseudonymized before being transferred to the centralized database. The pseudonymized data are made available to task groups defined in Section 13.1.

Imaging – DICOM-RT data & MRI images
At each investigating centers, DICOM-RT files are extracted from the local planning systems and Picture Archiving and Communication Systems, and transferred to the DICOM data repository by experienced staff after pseudonymization. Pseudonymization means here removal of all identifiers (patient's first and last names, patient's date of birth, doctors' identifications, administrative IDs, and free-text fields) done loccaly at the centre level, before any data transfer. The table of correspondence between the patient's identifiers, the study ID and the unique participant study ID is kept at the centre and authorized entity levels.

All DICOM data are stored and shared with encrypted cloud computing.

DICOM-RT data are downloaded by WP2 investigators to check the anatomical contouring done for treatment plan and add new delineated structures of interest for late effects analyses. DICOM data are downloaded by WP4 investigators for dose estimation to the whole-body and normal tissues of interest. The results of the DICOM analyses by WP4 (pseudonymised voxelized dose estimation and dose-volume metrics), together with the unique participant study ID, are transferred to the centralized database and made available to task groups for late effects analyses.

Neurovascular MRI images are downloaded by WP2 investigators for identification of imaging markers by the responsible neuroradiologist and radiation oncologist (see Section 13.1). The identified imaging markers are then uploaded into the centralized database and made available to the neurovascular task group.

Linkage to External Registries and Databases
Personal identifiers necessary for linkage are securely sent by email using Gnu Privacy Guard (GnuPG) encryption – or a similar encryption system – to the external registries, either by the investigating centre or by authorized entities (i.e. Inserm unit 1018 research group "Radiation Epidemiology", for the two French centers only), depending on the country. The personal identifiers are associated with a unique "request" ID, which shall be different than the Harmonic study participant ID and shall be different for all requests of linkage with external registries and databases, to ensure a satisfying level of data protection. The data extracted from the external registries are received, together with the "request" ID (no personal identifiers) by email using GnuPG encryption– or a similar encryption system –, or any other channel defined by the data provider having an equal or higher security level.

After matching the "request" ID with the Harmonic study participant ID, the pseudoanonymised results of the linkage are then included in the centralized core database. All transfer of personal data from and to registry or database shall be done in accordance to European and national/regional data protection directives and law. Access to the results of the linkage is strictly limited to authorised project personnel.

Centralized database management

Developpment and Setting-up

The e-CRF and the database will be developed through RedCap (https://www.project-redcap.org/) by an experienced data manager with the help of all investigators, and is stored on a local, secured servor at Inserm 1018, Centre de recherche en Epidémiologie et Santé des Populations, Hôpital Universitaire Paul-Brousse, 6 avenue Paul Vaillant Couturier, 94800 Villejuif, France.

The e-CRF are as similar as possible to the pre-existing local registry or database used in the participating radiotherapy departments for pediatric cancer patients, to allow automatic data transfer to the centralized database whenever it is possible.

Access and editing rights

Different user profiles with specific access and editing rights are defined:

- **investigating center**: access to structured data of own patients, which were either uploaded by the center itself or by a task group (e.g. dose estimation, biomarkers), and editing rights;
- **task group**: access to the data necessary to task-specific analyses - no editing rights but can upload data (e.g. dose estimation, biomarkers);
- **WP coordinators**: access to all data, patient workflow and dashboard - no editing rights;
- **project coordinator**: access to patient workflow and dashboard - no access to patient data, no editing rights;
- **sponsor**: access to patient workflow and dashboard; no access to patient data (except in case of audits); no editing rights.

Among partners, data access is limited to researchers dealing with data analysis or project management only.

Data and Material Transfer

Transfer of data or material between partners (or with linked third parties under the consortium agreement) is performed after data/material transfer agreements are signed. Templates of these agreements are included in the consortium agreement. It is reminded that:

- Pseudonymization is implemented as a general standard meaning that all data and biological material obtained in the framework of the project are identified through a unique participant study ID which link all basic data required for the study. The master key file linking this ID with personal identifiers is maintained, at the center and authorized entity levels, in an encrypted file with limited and secured access;
- Any personal and biological data transfer (except for linkage to external registries and databases) is done only after anonymization;
- All files containing personal data are stored in encrypted and password-locked files. Access to these files is limited to authorized project personnel;
- All project personnel is trained in the importance of confidentiality of individual records and required to sign a confidentiality agreement;
- All data transfers are completed using secured servers.

For the purpose of future national, European or international research studies (see Section 3.2), part of pseudonymized data and/or biological samples that are collected within the HARMONIC project may be transferred in the framework of other research relating to the evaluation of late outcomes of cancer treatments for management of paediatric cancers to other organizations or institutions which are located in the countries of the investigating centers or abroad, under the provision of the national laws and regulations, and the HARMONIC grant agreement, consortium agreement, Data Management Plan, and children and parent(s)/guardian(s) information and consent/assent forms.

12.4 Retention of Data Documentation

Research documents are archived in accordance with current regulations.

The sponsor and the investigators shall keep the research documents, which are specific to them for a period of X years.

No displacement or destruction may be made without the consent of the sponsor. At the end of the prescribed archiving period, the sponsor will be consulted for destruction.

All data, documents and reports may be audited or inspected.

13 STATISTICAL ANALYSES

Due to the nature and the duration of the project, it is not possible to anticipate all the analyses which will be performed on health and social outcomes associated with characteristics of radiotherapy and other cancer treatments. The present section details the analyses which are planned within the 5-year funding of the research by the European Commission.

13.1 Persons Responsible of the Statistical Analyses

Table 14. Persons Responsible of Task-Specific Statistical Analyses

Tasks	Information on Persons Responsible of the Statistical Analyses		
	Full names	Institute	Qualification
Endocrine dysfunctions	Beate Timmermann	UKEssen	Pediatric radiation oncologist
Cardiovascular diseases	Stéphanie Bolle Brice Fresneau Nadia Haddy	GR	Pediatric radiation oncologist Pediatric medical oncologist Epidemiologist
Neurovascular diseases	Yasmin Lassen Sanja Karabegovic	AUH	Pediatric radiation oncologist Neurovascular radiologist
Second primary cancers	Karin Haustermans Neige Journy	KUL Inserm	Pediatric radiation oncologist Epidemiologist
HRQoL and academic achievement	Agnès Dumas Juliette Thariat	Inserm CRFB	Sociologist Pediatric radiation oncologist
Biomarkers of vascular diseases and carcinogenesis			
○ RPPA	Stéphanie Bolle Brice Fresneau Nadia Haddy	GR	Pediatric radiation oncologist Pediatric medical oncologist Epidemiologist
○ Inflammatory and oxidative stress markers	Siamak Haghdoost	SU	Biologist, Toxicologist
○ Markers of carcinogenesis	Maria Grazia Andreas-si	CNR	Biologist

13.2 Sample Size

Table 15. Expected Numbers of Participants Included in Task-Specific Statistical Analyses as of 31 May 2023

Tasks	Expected Numbers of Participants Included in each Task by 31.05.2023
Endocrine dysfunctions	1600
Cardiovascular toxicities	100
Neurovascular damages	150
Second primary cancers	2670
HRQoL and academic achievement	1300
Biomarkers of vascular diseases and carcinogenesis	250

13.3 Statistical Analysis Plan

Unless otherwise stated, risk parameters will be estimated using maximum likelihood methods, and the statistical significance of risk differences or ratios will be assessed considering a two-sided alpha level of 0.05. Simple or multiple imputation of missing values will be considered for adjustment factors with <5% of missing values.

13.3.1 Endocrine dysfunctions

This task will quantify the dose- and volume-effects of radiation doses on pituitary and hypothalamic structures as well as on the thyroid gland in children up to the age 18 years who received EBRT in the CNS, head and neck region, upper thoracic aperture or cervical spine. The analyses will provide dose-volume risk estimates for photon and proton therapy. We will investigate modifying factors (e.g., age at exposure, genetic predispositions, comorbidities, and medications) that potentially underlie differences in individual radio sensitivity for endocrine dysfunction.

13.3.2 Cardiovascular diseases

The analyses will be conducted among individuals treated with mediastinal/chest/pulmonary irradiation at age <22 years, to investigate the associations between radiation dose-volume parameters for the heart and cardiac substructures and repeated measures of serum and imaging markers, while accouting for demographic and clinical data. Firstly, a classical analysis using paired T-test and/or Wilcoxon signed rank test will be used to compare clinical data and biomakers at baseline and each time point after irradiation. Secondly, based on repeated analysis at different timepoints, unsupervised analyses (e.g. group-based trajectory models) will be used to identify clusters of individuals who have similar trends of biomarkers over time. Thirdly, mixed models will be used to quantify the association between dosimetric indicators and biomarkers at different timepoints. Last, the data will be analysed as differences in biomarkers between each follow-up timepoint and baseline. The differences in biomarkers at follow-up vs. baseline timepoints will be fitted by general linear models as a function of dosimetric indicators (dose, volume and beam quality), while controlling for the effect of potential confounders including sex, age chemotherapy doses and others. More specific data analysis can be applied according to specific scientific questions.

13.3.3 Neurovascular diseases

The neurovascular task will focus on one side on neurovascular changes in small and large vessels after radiotherapy with the aim to identify imaging markers in relation to the clinical neurovascular diseases or symptoms. The other focus will be to investigate dose-volume relationships to the whole brain and to neurovascular substructures and how they relate to the imaging changes and clinical symptoms and to the employed radiation therapy techniques. If possible depending on the sample size, these data will be correlated to the serological neurovascular data and quality of life data using univariate and multivariate analysis, taking time into account. The analyses will be conducted among children aged < 18 years treated for a brain tumor with radiotherapy. Re-irradiation cases will be excluded.

13.3.4 Second primary cancers

This task will assess the associations between normal tissue radiation factors (i.e. total dose, dose fractionation, irradiated volume of the organ, beam quality) and site- and histology-specific cancer incidence, while accouting for sex, attained age, time since exposure and other confounding factors. The following cancer sites will be primarily investigated: CNS, thyroid, breast, lung, gastrointestinal organs and tracts, soft tissues, bones, and genital organs and tracts. However, investigations for other cancer sites are not excluded. We will also consider the stage at, and methods of second cancer diagnosis to detect potential surveillance biaises.

A wide range of factors which may bias or modify the radiation-risk relationship will be considered:

- systemic cancer treatments: chemotherapy, immunotherapy, hormonal therapy, targeted therapy, surgery
- clinical factors: family cancer history, genetic syndromes, comorbidities (e.g. overweight/obesity, vascular disease), non-cancer medications (e.g. hormone replacement therapy, contraception)
- lifestyle characteristics: smoking history, alcohol consumption, drug consumption, physical activity/sedentariness
- hormonal factors: sex-specific factors (e.g. age at puberty), comorbidities, treatments

The analyses will be perfomed using logistic regression or proportional hazards models, where the risk of disease is modelled as a function of summarized dose metrics for the whole organ of interest (e.g. mean dose, volume irradiated at ≥x Gy or x_1-x_2 Gy), or estimated dose values to each voxel of the organ (with different contributions of beam qualities). Radiation effect modifications by the above-mentioned factors will be tested as interaction terms in the risk models, or subgroup analyses to investigate potential determinants of individual susceptibility to second primary cancers.

Comparison of risks in patient groups treated with different EBRT techniques will require preliminary analyses of the clinical and other factors related to the likelihood to receive one or another EBRT technique (see Section 1.2.5). Firstly, we will review referral guidelines. Secondly, we will compare clinical and socioeconomic characteristics of paediatric patients treated with different EBRT techniques in the HARMONIC database, but also in other external databases which are representatives of the general population (e.g. SNIIRAM for France[12]). These preliminary analyses will enable us characterizing and quantifying the presumed indication bias in comparative studies, and defining appropriate statistical methods for dose-volume-risk analyses accounting for indication bias (i.e. subgroup, adjusted, and/or individually matched-pair analyses).

Given the long latency time between radiation exposure and radiation-related increased risks of solid cancers, the analyses on second solid primary cancers will be performed once we reach a minimal median follow-up time in the study population of about 10 years, and register sufficient number of cases.

13.3.5 HRQoL and academic achievement

The analyses will characterize longitudinal trajectories of HRQoL (using mixed linear models), comparison of scores between parent-proxy and child/adolescent reports, and scores in paediatric patients treated with modern radiotherapy techniques compared to population norms from the different countries (Dumas 2016). We will identify clinical and socioeconomic factors associated with HRQoL and academic achievement.

13.3.6 Biomarkers of vascular diseases, carcinogenesis, inflammatory and oxidative stress markers

Quality control and bioinformatics analysis

A standard quality control are performed for all biomarkers data. Proteins and miRNAs expression are analyzed (in triplicate) and normalized using specific bioinformatics methods (e.g. NormaCurve) and softwares (e.g. GeneSpring, STRING, INGENUITY). At each time point, one value for each miRNA or protein of interest are generated. The values are used for further statistical analysis. Bioinformatic analysis are also used to construct a map of gene-protein-miRNA interactions by using specific target prediction softwares and pathway enrichment analyses. For RPPA, the NormaCurve method will be used for data quantification and normalization [93]. This method includes a normalization for (i) background fluorescence, (ii) variation in the total amount of spotted protein and (iii) spatial bias on the arrays. The values are used to compare the expression level of the proteins of interest between the samples. In brief, for each spot the raw fluorescent signal of the proteins is corrected with the fluores-

[12] access pending authorisations by the competent regulatory authorities.

cent signal of the negative control (signal obtained after incubating an array without antibody targeting the protein of interest). This corrected signal is then divided by the total amount of spotted protein, corresponding to the normalized signal. Finally, the normalized signals of all the proteins are scaled according to the median.

Statistical analyses

In descriptive analyses, patient data will be expressed as mean ± standard deviation (normally distributed data), median and inter-quartile range (non-normally distributed data) or percent frequency (categorical data). Characteristics between two groups will be compared by $\chi 2$ test for categorical variables and two-sample T-test for quantitative variables. Comparison of 3 means will be performed by ANOVA test. Non-parametric data will be analyzed using Mann-Whitney or Kruskal-Wallis test followed by Dunn's post-test as appropriate. Exploratory analysis including unsupervised clustering and principal component analysis will be performed to cluster patients according to biomarker expression. Other unsupervised analyses (e.g. group-based trajectory models) will be conducted to identify clusters of individuals following similar changes of biomarkers over the time taking into account all time points will be performed. A mixed model will be used to study the association between different biomarkers at differ-ent time points and the dosimetric indicator (dose, volume and beam quality). Last, differences in biomarker value between a follow-up timepoint and baseline will be fitted as a function of dosimetric indicators (dose, volume and beam quality) using general linear models, with adjustment for potential confounders. Finally, a pathway analysis according to the different parameters will be performed. Statistical significance for all analyses will be assessed using a two-sided alpha level of 0.05 with adjustment for multiple comparisons using Bonferroni and/or False Discovery Rate. More specific data analysis can be applied according to specific scientific questions.

14 CONFIDENTIALITY

14.1 Conditions of respect for confidentiality with respect to persons

Pseudonymization is implemented as a general standard in the framework of the project. Each participant is given a unique participant study ID ("Study ID") without indication of the family or first name or, other personal data or number (such as social security or hospital number) which could allow the identification of the subject. All collected records, in any form, are identified through this code.
The study ID is created as a 14-digit number:

- Country code (3-digit number)
- Center code (3-digit number)
- Respondant code, i.e. participant / mother / father / guardian (1-digit number)
- Study subject code (7-digit number), which is randomly attributed by the data manager in charge of the centralized database

All the persons, including investigators, having access to the personal data are subject to the obligation of professional secrecy.

No identifying information is used in any publication or presentations.

14.2 Conditions of respect for confidentiality with respect to the research

A direct access to clinical and source data is provided in case of sponsor's monitoring and audit or inspection of competent regulatory authorities. All reasonable precautions within the constraints of the applicable regulatory requirement(s) are taken to maintain the confidentiality of subjects' identities and sponsor's proprietary information.

15 COMMUNICATION

Communication is subject to any commitments made by the HARMONIC consortium partners and any regulatory obligations.

In order to fulfill its sponsor's responsibilities, the present research will be registered on public websites: www.clinicaltrials.gov and www.clinicaltrialsregister.eu.

15.1 Publication of the Research Results

The success of the research project will be judged in part by the number and quality of its scientific publications and presentations. A list of main foreseen papers has been identified to date by the HARMONIC Consortium and additional papers (focusing on more specific issues) will also be published in the framework of the research, in accordance with HARMONIC publication policy.

The results are published after final analysis in the form of scientific articles in peer-reviewed journals, and presentations at national and international conferences. Results will be published in an agregated format whitout possibility to identify particpants identity. Any publication or communication (oral and written) of the results will meet the requirements of the Publication Policy defined by the HARMONIC Consortium and shall respect the international recommendations: "Uniforms Requirements for Manuscripts Submitted to Biomedical Journals" (http://www.cma.ca/publications/mwc/uniform.htm). Authorship must be based on the International Committee of Medical Journal Editors (ICMJE) criteria. All publications must follow the rules contained in the publication charter defined by AVIESAN.

All publications shall include the following statements:

- "The HARMONIC project (Health effects of cArdiac fluoRoscopy and MOderN radIotherapy in paediatriCs) has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 847707".
- */Ethics statement /*/This study is part of clinical trial ****CXX-XX** sponsored by Inserm, and under the equal responsability of AUH and UK Essen. It was granted approval by the local/national Ethics Committee or "Comité de Protection des Personnes" on ---****DATE**---, and registered in a public trials registry (****CT XXXX**).
  All study participants gave their informed, written consent to participation, in line with national legal guidelines.

The following statements should be also included to acknowledge additional funding sources:

- Complementary funding was received from...
- The X country part of the study was funded by ...
- In X country, complementary funding was received from a grant with the ...
- In X country, funding was received from ...
- The original X country cohort study was funded by... Complementary funding for the HARMONIC funded extension was received from...

Please refer to Harmonic publication policy for a detailed description.

All data collected remain under the sole control, management, custody and responsibility of the sponsor or the party collecting the data where there is no sponsor needed, and shall not be communicated to any third party without a Data/Material transfer agreement signed between the sponsor/party and the recipent. The detailed rules applying to research results' ownership are defined in the HARMONIC consortium agreement.

15.2 Reporting of the Study Progress and Final Report

The investigators will give full account of the study progress and results to the project coordinator, through the deliverables (which will be transmitted to the European Commission) listed in the grant agreement and approved by all investigators, and whenever they are requested by the project coordinator.

Once the research has ended, a final report of the results and a final summary report shall be provided to the sponsor within one year of the completion of the research in all the countries in which it has been carried out, in compliance with the national regulations.

The final report of the research is a written document, sufficiently detailed to allow an understanding of the research process and an objective judgment on the quality of the data of the research. It is prepared, in collaboration, by the research coordinator and submitted to all investigators for their review and approval. Once a consensus is reached, the final version is endorsed by the signature of each investigator and made available to the sponsor. This final report will be made available to the French Agency for the Safety of Health Products upon its request.

The final summary report shall include a summary of the results drawn up in accordance with the reference plan of the competent authority. The summary shall be validated and forwarded by the sponsor, or the responsible party where there is no sponsor, to the competent authority and the Research Ethical Committees in accordance with the type of research. This transmission must be carried out within one year following the end of the research in all the countries where it has been carried out.

15.3 Information of the Participants on the Research Results

Upon completion of the research, all study participants of the prospective part shall have the right to be informed of the overall results of the research pending request, in accordance with the procedures to be described to him or her in the information form. The overall results will also be publicly made available on the study website maintained by ISGlobal (www.xxxx).

15.4 Information of the Participants on their Personal Data during and after Research

General dispositions

Study participants can request information on their personal data at any time, through a simple request to a study investigator. Any medical information can be disclosed to the requesting individual only by a medical doctor who participates in the research. Any clinical or biological (clinically relevant or not) findings discovered during the research will be disclosed only through the medical doctor (oncologist, general practitioner, or other) who was mentioned by the requesting individual.

Incidental findings

All clinical procedures (blood test, hormonal measurements, imaging procedures, questionnaire) done will be reviewed by the referent medical doctor who will contact the participant / mother-father or legal tutor in case of any incidental finding. We will follow the general recommendations on disclosing incidental findings proposed by Anastasova et al (2013) Communication of results and disclosure of incidental findings in longitudinal paediatric research.

15.5 Press Communication

A dedicated communication working package has been established for the HARMONIC project, therefore it will take the lead on the communication activities. The main objective of its communication strategy is to maximise the visibility and impact of the project by 1) Developing communication material and tools 2) Disseminating Harmonic's aims and results throughout the entire course of the project to different target audiences, including scientists and medical and patient associations 3) Sharing resulting guidelines/recommendations with the medical and radioprotection community 4) Engaging relevant stakeholders at the local, national and international level to ensure exploitation of results beyond the project's lifetime.

Given the delicate nature of the project's topic (potential late effects of cancer treatements in paediatric patients), the communication strategy will focus on informing and engaging the medical and radioprotection communities and associations throughout the project duration, rather than on the general

public. Therefore, social media will be used only when justified, and at the end of the project when results are available and key messages can be communicated to wider audiences. Press releases for local, national and international media will be considered when relevant results become available and justify a wider reach. The design and content of these materials will be coordinated by ISGlobal with the approval of all partners. Investigators will be encouraged to submit abstracts for meetings and conferences in order to show the scientific community all information resulting from the HARMONIC project. The lead responsible author and co-authors are free to choose any national or international meeting or conference which may be interesting for showing results from the HARMONIC project. Communication with patients will be carried out mainly through the medical community. Please refer to Harmonic communication and stakeholder engagement plan for a detailed description

The articles and abstracts, but also the oral communications from this research will be sent for information to the HARMONIC steering committee and Inserm (Clinical Research Centre and the Department of Scientific Information and Communications) prior to publication.

16 PROTECTION OF INDIVIDUALS AND PERSONAL DATA

16.1 Ethical Rational of the Study Protocol

This research is conducted in accordance with the applicable French, Belgian, German and Danish laws and requirements, European authorities and with the Declaration of Helsinki.

No benefits for the individual research participants are expected. The objectif is the demonstration of benefits of society expected for other minors with optimisation of radiotherapy plans in paediatric patients by reducing late toxicities of radiotherapy.

Expected risks and constraints are low.

16.2 Adequacy of the investigating centers

Since participants are included at their national treating centers and the research biological samples are taken and radiological images are realized at these centers, conditions relating to human, material and technical means are ensured. However, should an incident occur, participants would benefit from all the human and technical means of the hospital of each participating centers to ensure their safety in accordance with the applicable hygiene and safety rules and with respect for the participants' integrity.

16.3 Ethical and Regulatory Provisions

The research shall be carried out in accordance with the French, Belgian, German and Danish laws in force, in particular:

- the provisions relating to research involving the human person provided for in Articles L 1121-1 et seq. of the French Code of Public Health, the laws of Bioethics, the law of Informatique and Libertés;
- the Belgian Law of 7 May 2004 concerning experiments on the human person and the Law of 7 May 2017, the Advisory Comité for Bio-Ethics and the National Counsel of the ‘Order der artsen’, the protection of the common good, safety, dignity, rights and privacy of patients/healthy volunteers (human subjects) participating in a clinical trial; the Belgian Law of 30 July 2018 on the protection of natural persons with regard to the processing of personal data;
- the Danish Act concerning the processing of personal data and health law;
- the German applicable federal and state Law as well as the medical professional code of conduct;
- the Swedish Dataskyddsförordningen, which implements the EU General Data Protection Regulation;
- the Italian applicable legislation, including the Legislative Decree 24 June 2003;

- the general regulation on data protection, the declaration of Helsinki, and the present protocol.

The investigator undertakes to conduct the research in accordance with these ethical and regulatory requirements. The investigator is aware that all documents and research data may be subject to audits and inspections carried out in accordance with professional standards and without the possibility of medical confidentiality being waived.

16.4 National and Local Ethical Committees

Prior to the conduct of the research, the protocol shall be submitted to the institutional review board (IRB) / independent ethics committee (IEC) in compliance with the applicable laws and requirements of each participating country (Table 16). Any necessary information should be provided to IRB/IEC.

The research should not start prior the IRB/IEC's written approval/favourable opinion, subject to any other compulsory authorisation necessary before the beginning of the research.

The IRB/IEC's written approval/favourable opinion should mention the title and the sponsor's protocol code number, the documents reviewed and approuved as well as the date of examination and the list of the members of the IRB/IEC's who were present.

The sponsor should inform the IRB/IEC's of any subsequent amendment accordingly to each participating country laws and requirements.

Table 16. List of the IRB/IEC to which the protocol shall be submitted

Country	Investigating Center	Competent IRB/IEC
Belgium	KUL	Ethics Committee Research UZ/KU Leuven
Denmark	AUH	Research Ethics Committee of the Central Denmark Region
France	GR	Will be apointed randomly from among 39 Research Ethics Committee
France	CRFB	
Germany	UK Essen	Ethics committee of the University Duisburg-Essen

16.5 Insurance

Inserm, as a sponsor of the study (for centers in France and Belgium only), subscrided for every country throughout the duration of the research a civil liability insurance with the following insurance policy number XXX, in compliance with applicable legal and regulatory provisions and according to the typology of the research.

17 COORDINATION AND GOVERNANCE

17.1 Independent Monitoring Committee

Not applicable

17.2 HARMONIC Management Structure

The management structure of the project is elaborated to ensure successful achievements of the main objectives of the project. The management structure allows clear identification of responsibilities, optimisation of communication between the partners, with the European Commission and with medical and RP communities. It is based on rules and regulations that will also be described and agreed upon in the consortium agreement. It is framed according to three inter-linked objectives:

- To implement decision-making, quality control and conflict resolution mechanisms;
- To provide timely and efficient contractual, scientific, financial and administrative coordination of the project;
- To ensure timely and high-quality execution of the project.

In order to facilitate the successful conduct of the project, the structure described below and summarized in Figure 5 has been put in place.

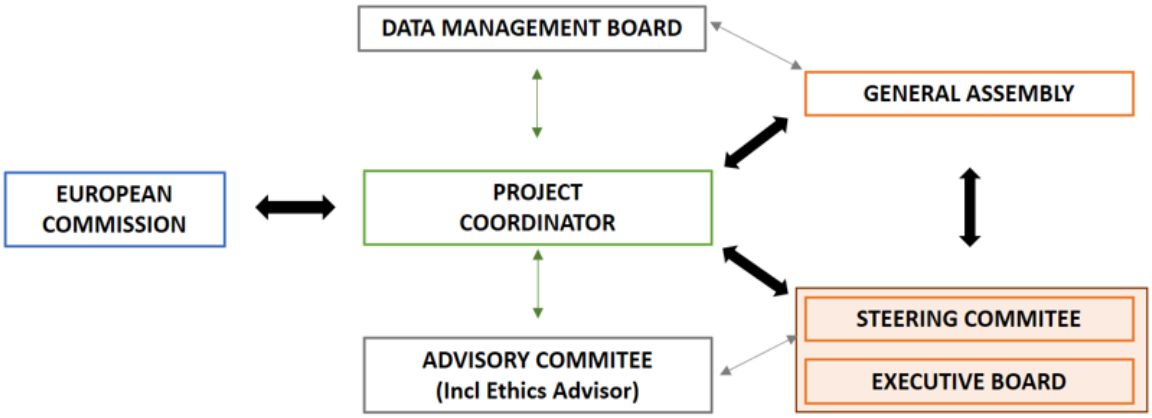


Figure 5: Management structure of HARMONIC

The Project Coordinator, ISGlobal (Dr. Isabelle Thierry-Chef), operates as an intermediary between the Consortium and the European Commission. The coordinator of the project is responsible for scientific coordination of the project (ensuring smooth conduct of the project, including coordination and follow-up of the work, timely provision of results and adherence to European Code of Conduct for Research Integrity, including monitoring ethics issues arising in the project), the contractual and financial management of the HARMONIC project as well as for overall monitoring of compliance with the project work plan. The Coordinator is the legal entity acting as the intermediary between the Parties and the Funding Authority. The coordinator shall, in addition to its responsibilities as a party, perform the tasks assigned to it as described in the grant agreement and this consortium agreement. A project manager is responsible for the day-to-day management of the project (financial and contractual issues, managerial, organisational, and administrative matters of the project). The project manager in collaboration with coordinator and steering committee is in charge of the project risk management, data management and quality control.

The General Assembly is composed of one representative of each partner institutes. The representatives are clinicians and experts in clinical specialities (radiation and medical oncologists, cardiologists, radiologists, medical physicists, endocrinologists, nurses, and psychologists) involved in paediatric care, and radiation research scientists (epidemiologists, nuclear physicist, biologists, sociologists, and radiation protection experts) in the countries where the study will be conducted. the general assembly shall be free to act on its own initiative to formulate proposals and take decisions in accordance with the procedures set out herein. in addition, all proposals made by the steering committee shall also be considered and decided upon by the general assembly. the general assembly shall have final, overall responsibility for the scientific decisions, and votes on major modifications of the work plan, possible changes in partners, as well as unresolved management issues.

The Steering Committee is composed of WP leaders and assists the coordinator in the project management and coordination. It comprises: Dr Isabelle Thierry-Chef (ISGlobal), Prof Beate Timmermann (UK Essen), Dr Neige Journy (INSERM), Prof Mark Pearce (UNEW), Dr Marie-Odile Bernier (IRSN), Dr Jérémie Dabin (SCK-CEN), Dr Siamak Haghdoost, (SU), Dr Adelaida Sarukhan (ISGlobal).

The Executive Board is composed of WP leaders and task leaders. They coordinate the work in a specific WP and are responsible for the planning, monitoring and technical reporting of the WP progress. They are responsible for ensuring deliverables and milestones are on time. The executive board will make day-to-day decisions at the technical level, in consultation with the steering committee if needed.

The Data Management Board constitutes of one representative person appointed by of each Party where Data is collected, stored or used. Members of the Data Management Board are responsible for data management and ensure, throughout the project, that data are stored securely and according to international standards, both from a technical and ethical standpoint (de-identification, pseudomization).

The Advisory Committee is consultative and consists of world-renowned experts in the fields of oncology, cardiology, imaging, radiobiology, dosimetry, medical physics, radiation protection and radiation epidemiology, psychology/sociology. The Advisory Committee shall assist and facilitate the decisions made by the General Assembly as well as review and advise on the scientific and clinical aspects of the project. The Coordinator will on behalf of the Consortium ensure that a non-disclosure agreement is executed between all parties and each Advisory Committee member. We also value representation of an international patient organisation and participation of members of existing national registries for consultation. The ethics advisory is a member of the advisory committee with reinforced links with project coordinator and data management board.

WPs and Tasks

The scientific activities are structured as WPs and WP-specific tasks (Figure 1). WP and Task coordinators' responsabilities are dezatiled in the HARMONIC grant agreement.

Engagement with Stakeholders

The Consortium also values representation of an international patient organisation and participation of members of existing national registries for consultation:

- Platforms in radiation protection research;
- Medical community;
- International, european and national medical scientific societies;
- Healthcare authorities concerned with radiation protection;
- International and European organisations with an interest in radiation protection;
- Radiation Protection associations.

18 QUALITY ASSURANCE

18.1 Description

At Harmonic management bodies' level, the quality assurance-related tasks include:

- Follow-up on the description of action to communicate upcoming commitments, and the early identification of deviations to promote corrective actions;
- On-going communication with the coordinator and WP leaders to monitor progress of tasks, deliverables, and milestones;
- Facilitate interaction between partners (and also external advisors in some instances) to cross-check outputs, deliverables, and reporting, and ensure high-level materials are produced.

Please refer to the Harmonic quality assurance and risk management plan for a detailed description. To ensure that abstracts and publications based on HARMONIC project material are accurate and objective, and do not compromise the scientific integrity of this collective project, a publication policy has been established.

At sponsor level, the role of the quality assurance is to guarantee the safety of the research participants and to ensure the credibility of the data obtained from such research and their recognition by the medical and scientific community.

The investigator guarantees the quality of how the research is conducted. In France and Belgium, the research is framed by Inserm' standard operating procedures (SOP) and by research-specific SOP if necessary. All research-specific SOP must be validated by the sponsor. In all countries, the research is framed by International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practices.

18.2 Monitoring

Not applicable

19 SUBSTANTIAL AMENDMENTS TO THE PROTOCOL

Any substantial request to amend the authorized research project must be submitted by the coordinating investigator for advice to the sponsor.

After sponsor's favorable opinion, the sponsor shall implement the regulatory administrative procedures necessary for obtaining the approval of these substantial amendments by the Research Ethics Committee and/or the competent authority in the applicable country(ies).

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21 LIST OF ANNEXES

Annex 1. Study Synopsis

Annex 1-A. Study Synopsis (EN version)

Annex 1-B. Study Synopsis (FR version)

Annex 2. List of Participating Centers and Contacts of the Principal Investigators

Annex 3. Letters of Support from European and International Radiation Oncology and Cancer Societies

Annex 4. PedsQL™ Questionnaires (Generic Core Scales, Multidimensional Fatigue Scale)

Annex 5. Academic Achievement Questionnaire

Annex 5-A. Academic Achievement Questionnaire (EN version)

Annex 5-B. Academic Achievement Questionnaire (FR version)

Annex 6. Individual Information, Consent and Assent Forms

Annex 6-A. Individual Information and Assent Form, Children aged 7-11 years (FR version)

Annex 6-B. Individual Information and Assent Form, Children aged 12-17 years (FR version)

Annex 6-C. Individual Information and Consent Form, Major Participants (FR version)

Annex 6-D. Individual Information and Consent Form, Parents/Guardians (FR version)

Annex 7. Detailed Protocol of the Dosimetry Analyses (WP4)

Annex 8. Detailed Protocol of the Biological Analyses (WP5)

Annex 9. HARMONIC Data Management Plan