This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No. 847707

STANDARD OPERATING PROCEDURES

14/04/2021 – V.1.0

Harmonic
Health effects of cardiac fluoroscopy and modern radiotherapy in paediatrics

Working document - for internal use only

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IX. Dosimetry WP4 – Data transfer to Aquilab		
To be prepared		
	Date:	
X. Data linkage		
To be prepared		
	Date:	

Updates to this document should be maintained throughout the life of the study to reflect any change in procedures that may arise.

Version History			
Version	**Date**	**Reason for change**	**Approved by**
1.0	**21.06.2021**	Creation of the SOP	Isabelle THIERRY-CHEF

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Applicable documents

WP2 Protocol v 1.0 (Deliverable 2.1)

The following Standard Operational Procedures (SOP) are enclosed for data and samples collection.

- SOP I Correspondence table and definition of the participant study ID (task leader Dr Neige Journy and Dr Thi Chiên Tran)
- SOP II Blood Sampling
- SOP III Blood and saliva sampling in WP5 Biology (Task leader Dr. Siamak Haghdoost)
- SOP IV Blood sampling and anthropometry in WP2 Task 2.2 Endocrine Dysfunctions (Task leader Prof Dr. Beate Timmermann)
- SOP V Cardiovascular toxicities Task 2.3 (Task leaders Dr. Stéphanie Bolle, Dr. Brice Fresneau, Dr. Nadia Haddy)
- SOP VI Neurovascular toxicities Task 2.4 (Task leader Dr. Yasmin Lassen)
- SOP VII Second cancer (Task Leader Dr. Neige Journy, Prof. Dr. Karin Haustermans)
- SOP VIII Quality of Life Task 2.6 (Task leader Agnès Dumas)
- SOP IX Dosimetry WP4 – Data transfer to Aquilab, will be provided after framework is established with Aquilab (Task leader Prof. Lorenzo Brualla). In the meantime, please make sure that the link to treatment planning is kept.
- SOP X Data linkage (Task leader Prof Dr. Beate Timmermann and Dr Neige Journy) This SOP will be prepared at a later stage and prior to first linkage in 2023.

An associated document with HARMONIC guidelines will be provided to help for data entry into the REDCap database.

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Participating centres

TABLE 1. List of participating centres and definition of abbreviation according to participating county

COUNTRY	INVESTIGATING CENTRE	ABBREVIATION FOR COUNTRY	PRINCIPAL INVESTIGATOR
Belgium	Department of Radiation Oncology, UZ Leuven	BEL_KUL	Prof Dr. Karin Haustermans
Denmark	Danish Centre for Particle Therapy | Aarhus Universityhospital	DNK_AUH	Dr. Yasmin Lassen
Germany	Universitätsklinikum Essen (AöR) Klinik für Partikeltherapie Westdeutsches Protonentherapiezentrum Essen (WPE) Partnerstandort im Deutschen Konsortium für Translationale Krebsforschung	DEU_ESSEN	Prof Dr. Beate Timmermann
France	Centre François Baclesse, Service Radiothérapie	FRA_CRFB	Prof Dr. Juliette Thariat
	Gustave Roussy, Department of Radiation Oncology	FRA_GR	Dr. Stéphanie Bolle

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I. Correspondence table and definition of the participant study ID

Within the Radiotherapy component of the Health Effects of CArdiac FluoRoscopy and MOderN Radiotherapy in PediatriCs project (HARMONIC-RT), devoted to evaluate the late health and social outcomes of modern external radiotherapy techniques using photons or protons in paediatric patients, some identifying data of participating patients will be collected (see

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). However, to ensure security and privacy of patients, no patient identifying data is allowed to be sent outside the investigating centre except for the linkage with existing external registries/databases (HARMONIC protocol v1.0 and SOP X – Data linkage). Therefore, pseudonymization is implemented as a general standard in the framework of the project.

The purpose of this Standard Operating Procedure (SOP I) is to define and provide instructions for the definition of a Participant Study ID and the creation of a correspondence table with patient identifying information, which is fundamental for the follow-up of the patients. It describes the pseudonymization procedure and the flow of identifying data and pseudo-identifying data in the project.

If there is any question or information needed, please contact:

NAME	ROLE	EMAIL
Neige JOURNY	WP2 co-leader	Neige.JOURNY@gustaveroussy.fr
Thi-Chiên TRAN	Project manager and database administrator	Thichien.TRAN@gustaveroussy.fr
Angela JACKSON	Database administrator	angela.jackson@inserm.fr

3.1. Correspondence table

To ensure not only patient security and privacy but also source data verification, a correspondence table must be created in each participating centre. It is mandatory to maintain the link between identifying data of the study participants and pseudo-anonymized data collected in the HARMONIC-RT study.

A template of the correspondence table is proposed to the participating centres by the HARMONIC data manager's team.

The template includes (Annex I.2):

- the identifying data which are recommended to be collected by the investigating centres for traceability and follow-up purposes;
- the identifying data which are recommended to be collected by the investigating centres for data linkage with external registries/databases (e.g., national cancer registry, national mortality registry);
- the pseudo-identifying ID at the centre level (**Study Subject code**)
- the pseudo-identifying ID at the HARMONIC Registry level (**Participant Study ID**).

The use of this template is not mandatory. The content of the correspondence table is at the discretion of the investigators. However, the template should be used as a guidance to ensure the correspondence between identifying data and the HARMONIC central clinical and dosimetry databases managed by INSERM in France and/or with the biobank located at Stockholm University in Sweden (Figure I.1) is maintained.

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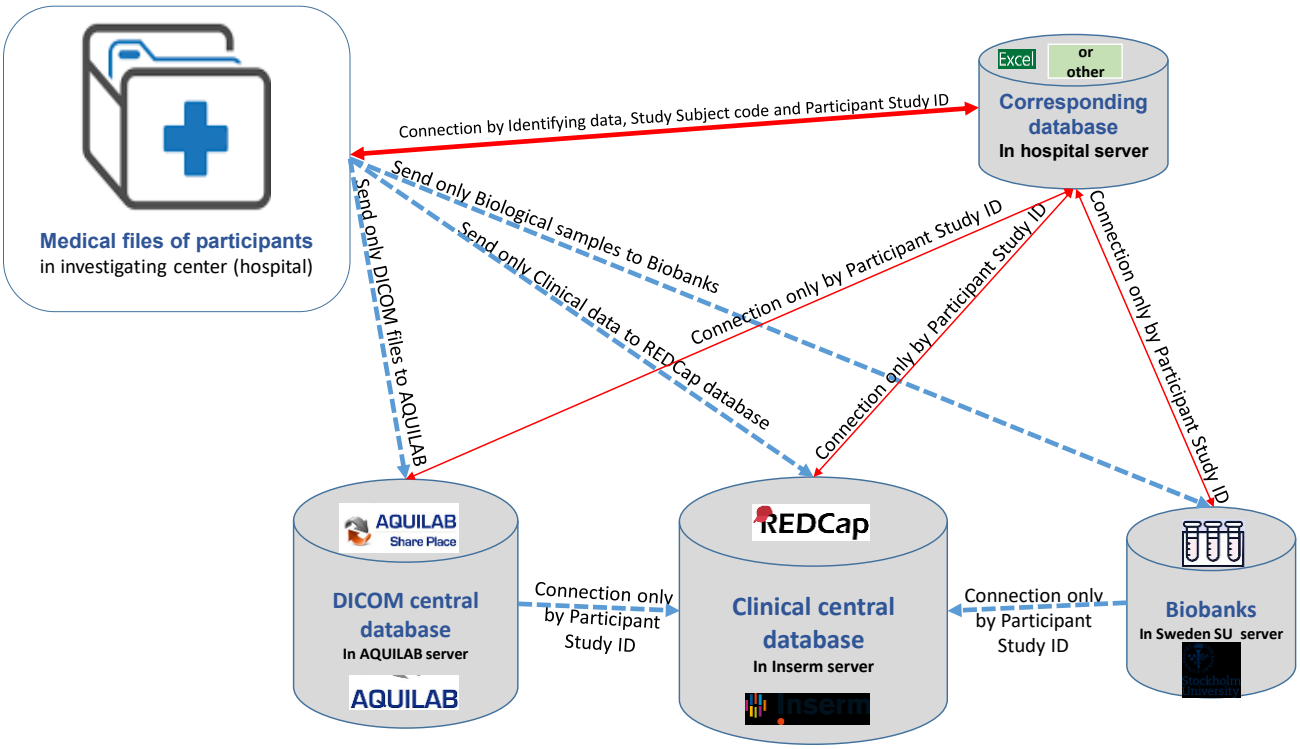

Figure I.1. Data flow within HARMONIC-RT

The correspondence table must contain the two pseudo data:

- **Study Subject code** **is a 6-digit pseudo-ID** (e.g, 000001, 000002, 210001...). **This code is unique, in each participating centre, and used only for patients included in the HARMONIC project**. It is defined and assigned by the principal investigator or her/his representative to the participants at their inclusion in the HARMONIC-RT study. It must be recorded by the investigators or her/his representative in the HARMONIC-RT Clinical central database through RedCap.
- **Participant Study ID** is a 12-digit unique pseudo-ID based on the Study Subject code. **Participant Study ID** is automatically provided by the REDCap interface when registering a new participant (See Section 3.2).

The principal investigator is responsible for the maintenance of the correspondence table at her/his centre.

The correspondence table is strictly kept locally at the investigating centre and can never be transmitted to the centralized database, other investigating centres or third parties, except the sponsor for monitoring purposes and authorized persons for the only purposes of long-term follow-up and linkage with external registries or databases. Access to the correspondence table is subject to the obligation of professional secrecy.

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3.2. Participant Study ID

Definition of Participant Study ID

According to the definition in HARMONIC protocol, the **Participant Study ID** contains a country code, a centre code and the Study subject code (see Annex I.1 and Section **Fejl! Henvisningskilde ikke fundet.**).

Each study participant must be given a unique **Participant Study ID (Registry level)** as soon as possible because all collected records, in any form, are only identified by this code through the correspondence table.

It is important to note that a patient will be considered as a participant in HARMONIC only when she/he obtained her/his unique **Participant Study ID**.

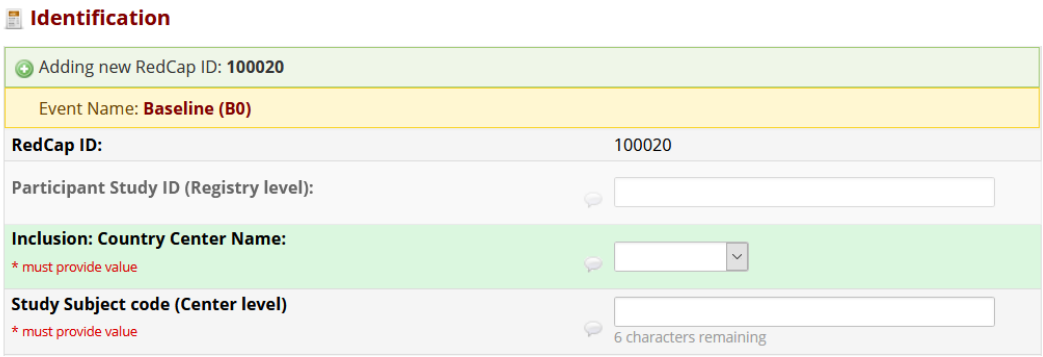
Creation of Participant Study ID

From REDCap, **Participant Study ID** will be obtained for each participant through **7 steps**:

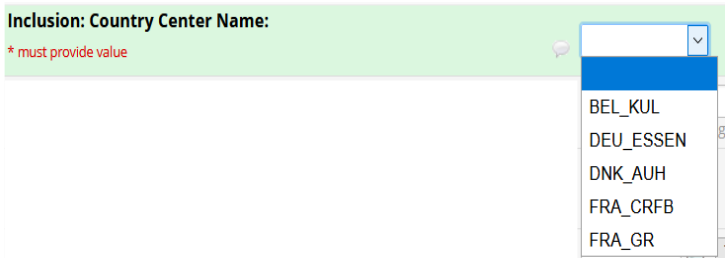
Step 1: Log into HARMONIC account

Step 2: Click at "**Add new record**"

Step 3: Open "**Identification"** form



Step 4: Click at "**Inclusion: Country Centre name"** then select the corresponding Country Centre name (**Fejl! Henvisningskilde ikke fundet.**)



Step 5: Enter 6 digit-number in "**Study Subject code (Centre level)"** of the participant. **This code is unique and used only for patients participating in HARMONIC project.**

Step 6: Click at **Save** to obtain automatically "**Participant Study ID (registry level)"** with 12-digit numbers.

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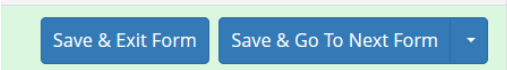


Step 7: **Participant Study ID (registry level)** must be registered in the correspondence table.

Example for creation of Participant Study ID

If the principal investigator assigns the number 000001 for the first patient included in Gustave Roussy Department of Radiation Oncology in France, the **Participant Study ID** will be obtained by ***7 steps:***

Step 1: Log into HARMONIC account

Step 2: Click at **"Add new record"**

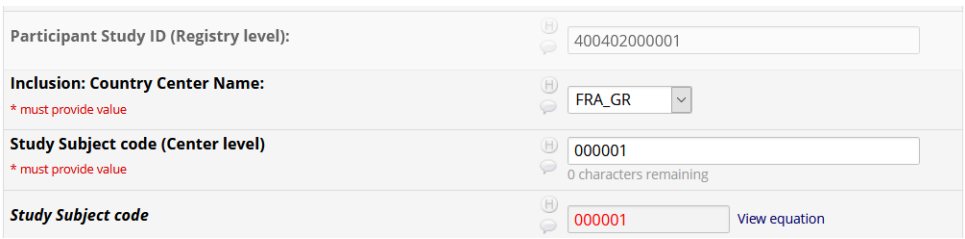
Step 3: Open **"Identification"** form

Step 4: click at ***"Inclusion: Country Centre name"*** then select **"FRA_GR"** (TABLE 1)

Step 5: Enter 000001 (6-digit number) in ***"Study Subject code (Centre level)"*** for the patient.

Step 6: Click at **Save.**

REDCap provide automatically number **400402000001** (with 12-degit numbers) as **Participant Study ID *(registry level)*** for the patient because **400402** is the code for Gustave Roussy centre in France (Annex I.1, 400 for France and 402 for Gustave Roussy) and **000001** is the Study subject code of the patient.



Step 7: Register the number **400402000001** as **Participant Study ID *(registry level)*** for the patient in the correspondence table (

Table).

Table I.1. Example of a correspondence table for Gustave Roussy

Study Subject code (Centre level) (6-digit number)	FULL NAME	DATE OF BIRTH (dd/mm/yyyy)	HOSPITAL ID	Participant Study ID (Registry level) (12-digit number)
000001	Marie BLANC	20/10/2018	200010760 MB	400402000001

Use of Participant Study ID

In the framework of HARMONIC project, no identifying data should be used outside of the investigating centre. In order to maintain the link, pseudo-identification such as **Study Subject**

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code (Centre level) and **Participant Study ID (registry level)** should be used in the DICOM files, biological samples or questionnaires while they are sent outside the investigating centres.

For each participating patient, the **Participant Study ID (registry level)** will be used as a correspondence code between her/his medical file with data collected in HARMONIC REDCap, DI-COM central database, biobanks or in questionnaires.

Results obtained from DICOM analyses, biological analysis or the questionnaires will be identified for each patient only by her/his **Participant Study ID (registry level)**.

The linkage will be done only using **Participant Study ID (registry level)** between HARMONIC REDCap and DICOM database, biobanks or questionnaires in paper.

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Correspondence table

Under responsible of principal investigator, a correspondence table must be created and kept up to date in each participating centre as it is the only way to link identifying data of the study participants with pseudo-anonymized data collected in the HARMONIC-RT study. In other word, it is only way to ensure the correspondence between participants and their data collected in the central HARMONIC clinical and dosimetry databases or with the biobank.

The correspondence table must contains:

- the identifying data which are recommended to be collected by the investigating centres for traceability and follow-up purposes;
- the identifying data which are recommended to be collected by the investigating centres for data linkage with external registries/databases (e.g., national cancer registry, national mortality registry)
- the pseudo-identifying ID at the centre level (**Study Subject code**)
- the pseudo-identifying ID at the HARMONIC Registry level (**Participant Study ID**).

Access to the correspondence table is subject to the obligation of professional secrecy

Participant Study ID

- **Participant Study ID (Registry level)** is a 12-digit unique pseudo-ID based on the **Study Subject code (Centre level)**. Participant Study ID is automatically provided by the REDCap interface when registering a new participant (See Section 3.2).
- **Study Subject code (Centre level)** is a 6-digit unique pseudo-ID of patient participated in HARMONIC study. It is defined and assigned by the principal investigator or her/his representative to the participants at their inclusion in the HARMONIC-RT study. It must be recorded by the investigators or her/his representative in the HARMONIC-RT Clinical central database through REDCap.

Each study participant must be given a unique **Participant Study ID (Registry level)** as soon as possible because all collected records, in any form, are only identified by this code through the correspondence table.

A patient will be considered as a participant in HARMONIC study only when she/he obtained her/his unique **Participant Study ID**.

No identifying data but only pseudo-identification such as **Study Subject code (Centre level)** and/or **Participant Study ID (registry level)** are allowed to fill out in the DICOM files or biological samples or questionnaires while they are sent outside the investigating centres.

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Annex I.1 List of country and centre codes in the HARMONIC project

INVESTIGATING COUNTRY		INVESTIGATING CENTRE		INCLUSION: COUNTRY CENTRE NAME (HARMONIC REDCAP)	INCLUSION: COUNTRY CENTRE CODE (HARMONIC REDCAP)
NAME	CODE	NAME	CODE		
Belgium	100	Department of Radiation Oncology UZ Leuven,	101	BEL_KUL	100101
Denmark	200	Danish Centre for Particle Therapy | Aarhus Universityhospital	201	DNK_AUH	200201
Germany	300	Universitätsklinikum Essen (AöR) Klinik für Partikeltherapie Westdeutsches Protonentherapiezentrum Essen (WPE) Partnerstandort im Deutschen Konsortium für Translationale Krebsforschung	301	DEU_ESSEN	300301
France	400	Centre François Baclesse Service Radiothérapie	401	FRA_CRFB	400401
		Gustave Roussy Department of Radiation Oncology	402	FRA_GR	400402

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Annex I.2 Template for the correspondence table

N°	DATA TO BE KEPT AT THE INCLUSION CENTRE LEVEL NO DATA TRANSFER EXCEPT FOR Study Subject Code and Participant Study ID
1	Study Subject Code (Centre level)
2	Participant Study ID (Registry level)
3	Referring institute
4	Referring MD
5	Participant's hospital number+++
6	Participant's full name
7	Participant's place of birth (country and city)
8	Participant's complete date of birth (dd/mm/yyyy)
9	Participant's full postal address
10	Participant's email address
11	Parent/Guardian's full postal address
12	Parent/Guardian's email address
13	Participant's Informed consent, date of informed consent (dd/mm/yyyy)
14	Participant's Consent - items
15	Parent/Guardian's Informed consent, date of informed consent (dd/mm/yyyy)
16	Parent/Guardian's Consent - items
17	Study withdrawal - reason (health status e.g, death, refusal, etc.)
18	Study withdrawal - date
19	Emigration information
20	Languages
21	National civil identification / health insurance number
22	Health insurance coverage/scheme

Items #3, 4, 9-13, 19 are useful for follow-up purposes

Items #14-18 are necessary to check the status of the patient of the study

Items #6-8, 21-22 are necessary for linkage with external registries (eg. To retrieve cancer information, vital status) if authorized

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II. Blood sampling

This chapter presents the standardized procedure for blood sampling affecting the clinics and centres that take blood samples on their own. The procedure is a recommendation according to the specifications of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). The main points listed should therefore be adapted to the respective clinic and department. Superfluous points should be deleted and specific points added.

1.1. General and Special

- **General / Inventory / Devices**

Equipment for blood sampling for **each patient**

Table II.2 Required Supplies: Blood sampling (General)

Amount	Equipment
1	Roller stool (for the examiner)
1	Hospital bed or blood collection chair with armrest (for the subject)
1	Armrest (washable)
1	Special waste container / Sharps disposal box (1 in use, 1 replacement)
1	Infectious waste biohazard bins
1	Tourniquet
2	Test tube rack (for the filled blood collection tubes)
1	Vomit bowl (for single use)
1	Radio controlled clock

- **Special supplies**

Table II.3 Required Supplies: Blood sampling (Special)

Amount	Supply
2	Winged blood collection set (butterfly devices) (different sizes)
1	Antiseptic skin disinfection spray before blood sampling
1	Surface disinfection (devices, arm rests, work surfaces)
1	Cellulose swabs with dispenser
2	Plaster
1	Pack of non-sterile gloves (different sizes)

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Amount	Supply
1	Package garbage bags
1	Pack of tissues
	Subject specific label set from the respective hospital/laboratory XY (adapt to local conditions!)

1.2. Inventory

— **General / Inventory / Devices**

- Refrigerator (4°C) with deep-freeze section (for freezer packs) for sample storage until analysis in the laboratory. Alternatively, suitable transport facilities can be provided. It must always be ensured that the cooling chain is not interrupted.

— **Special supplies**

- Tubes (according to the specifications of the respective laboratory) with cap

1.3. Documents

— Laminated paper labels for blood tubes with sample ID

→ CAVE: Partner participating in WP5

1.4. Information

— **Patient information**

- Local patient ID label is to be attached to the patient's medical record and matched with the name of the actual patient, before processing, in the direct interview of the patient.

— **Sample information**

- Available samples ID number.

2.1. Sample Preparation

Table II.3 summarizes the essential parameters in subject preparation and sampling and indicates the internationally recommended laboratory medical guidelines and their principles.

Table II.3 Recommendations on venous blood sampling – Pre-sampling and sampling

Parameter	Recommendation
Verify patient is fasting and properly prepared (sobriety)	For the lab-chemical analysis of hormones in blood, the fasting of patients is not necessary.
Time of blood sampling	Unitary in the same time window, usually in the morning, 7-9 am (consider the circadian variation)

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Parameter	Recommendation
Rest period in unchanged body position before blood collection	About 15 min
Body position during blood collection	The sitting position is recommended for outpatient blood collection
Duration of blood stasis before blood collection	Max. 1 min - open as soon as needle in vein
Blood collection technique	- Large volume cannula - No bathing of the arm in hot water - No prolonged searching/ 'poking' with the needle on of a job - No forced/rapid aspiration - No aspiration of para-venous blood - No repeated fist closure/pumping
Order of draw	First serum, then EDTA if required

2.2. Sample Collection – Examination Site

The room temperature should be comfortable (about 20°C-24°C). The blood collection chair can be bent over to form a bed, so that the test person can lie down in case of indisposition.

The material stock at each blood collection site should be checked daily before starting the blood collection. The blood collection area must always be kept thoroughly clean. Used material must always be disposed immediately in the appropriate containers. Clean the table, the armrest and, if necessary, the floor after each blood collection with surface disinfection and disposable tissues/cellulose.

Thorough hand washing or hand disinfection must be performed before and after each blood sampling. The examination site must be staffed by a technically experienced examiner who is experienced in blood collection. The qualification must be documented in a training and confirmed by the study management.

2.3. Sample Collection – Preparation

— Conversation with the patient:

- Explain your intention to the patient.
- Give the patient the opportunity to ask questions. Give him/her the impression that his/her questions will be taken seriously and answered competently.
- If the patient is a little anxious, take time, try to calm him/her down and convince him/her of the importance of the examination.
- Do not under any circumstances try to persuade the patient to take a blood sample.

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— Carefully check that the collection number of the blood collection tube matches the collection number in the patient record.

— Prepare the other material required for blood collection as listed in Table II.1.

2.4. Sample Processing

Profound knowledge regarding blood sampling is required. Detailed information about the process can be obtained in Annex II.2 .

Table II.4 Recommendations on Venous Blood Sampling – The Order of Steps

	Step	Strength of evidence
1.	Identify a patient	1C
2.	Verify patient is fasting and properly prepared (not necessary for hormone analysis)	1B
3.	Obtain supplies required for blood collection	2C
4.	Label/Identify tubes	1C
5.	Put on gloves	1C
6.	Apply tourniquet	1A
7.	Select venepuncture site	1B
8.	Clean sampling site	1B
9.	Puncture the vein	1A
10.	Draw first tube	1A
11.	Release the tourniquet	1A
12.	Gently invert the tube once (one full inversion)	1B
13.	Draw additional tubes following order of draw	1B
14.	Remove needle from the vein and activate safety feature	1A
15.	Dispose of the needle	1A
16.	Bandage the puncture site	1C
17.	Tell a patient to apply a gentle pressure for 5–10 min and not to bend the arm	1C
18.	Invert all tubes 4 times	1B
19.	Remove gloves	1A
20.	Advise patient to rest for 5 min and ensure bleeding has stopped before leaving the site of venous blood collection	1B

1A = Strong recommendation, high quality evidence; 1B = Strong recommendation, moderate quality evidence; 1C = Strong recommendation, low quality evidence; 2C = Weak recommendation, low quality evidence. [2]

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2.5. Post Sampling

After making sure that the patient is well, clean the workplace and accompany him/her to the next examination. Used swabs, cannula and other items are disposed into the appropriate containers. In order to minimize the risk of injury, it is mandatory to avoid recapping of protective caps on cannulas [6]. Finally, clean the armrest, work surface, blood collection chair or, if necessary, hospital bed with a surface disinfectant and disposable cloths. Thereby please pay attention to the local regulations. Throw away the used disposable cloths in the container assigned for this purpose. Please label the Monovettes and store the samples in a refrigerator or a suitable cooling medium until collection or transport to the laboratory.

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Annex II.1 Sample Processing

1. Search for the puncture site:

The sleeve is rolled up over the elbow, whereby the rolled-up sleeve must not cause any stasis. Pay attention to the stretched but relaxed position of the arm on the arm pad. Apply the tourniquet 7.5 - 10 cm above the venipuncture site. Palpate and follow the course of the veins several times with the forefinger. Blocked veins lack elasticity roll very easily. If the superficial veins are hardly visible, then you can ask the subject to make a fist. As a rule, however, fist closure should be avoided, especially repeated fist closures ('pumping'). Slight tapping of the vein with the forefinger/ middle finger several times causes a swelling of the vein. Lowering the arm causes the veins fill up more and more. In difficult exceptional cases, damp heat can also promote the emergence of the veins: Hold a towel under warm water and place it on the crook of the arm for several minutes. Heat application belongs to the control deviations, which shall be documented in the patient record!

As soon as you have found a suitable site for the puncture (but no later than after 1 minute), ease the tourniquet!

2. Cleaning and disinfection of the puncture site:

Spray the puncture site with the skin disinfection solution of your choice and let it take effect for 30 seconds. Optional: Clean the skin at the puncture site by rubbing once with a cellulose swab. Wait until the place is dry, in order to avoid possible hemolysis of the blood by the disinfectant which leads to changes in various blood values. If the venipuncture appears difficult, you may have to re-puncture the vein. In this case, the affected area must be treated again with disinfection.

3. Puncture:

Tighten the skin of the subject by pulling distally with the thumb alone or with thumb and forefinger. The wing cannula is kept in a position with the needle opening facing upwards. Use your eye to fix the area of the vein in which the needle tip should end. Then place the needle tip on the skin approximately half a needle length distally of this vein area. Then, with the needle in a vertical position, push through the skin with a gentle movement and then push the needle, which is now held flat, smoothly and evenly under the skin into the vein.

If blood flows into the tube, open the tourniquet immediately!

Place the serum (gel) Monovette on the Multi-Adapter, lock it by turning it clockwise and aspirate some blood as a sample. Should there be no blood flow, this usually means that the vein has been punctured. Pulling back the needle is no solution, because the vein wall is injured in two places and aspirating para-venous blood is strictly forbidden. This means that, in most cases, the needle must be inserted again, distally on the same or the other arm.

4. Filling the blood tubes (Monovettes):

Follow the filling sequence (first tubes without additives).

5. Remove the needle:

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Place a cellulose swab over the venipuncture site. Remove the needle quickly then immediately afterwards (i.e. not yet while pulling it out) exert pressure with your thumb on the puncture site.

Ask the patient to apply the swab firmly to the puncture site for a few minutes. The arm should not be bent at the elbow. Make sure that the bleeding has stopped and then cover the puncture site with a plaster.

If a haematoma develops at the puncture site or the patient complains of pain, a bandage can provide relief. Immediately throw the wing cannula into the container provided.

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Annex II.2 Central Venous Catheter Blood Draws - Discard

a) Required supplies

Amount	Supply
1-2	Non-sterile gloves
2	Packet of sterile compresses
1	If necessary Syringe 5 ml
2	10 ml PosiFlush XS syringe
1	Octenisept Spray
2	IN plugs
1	5 ml ampoule of Medusana heparin 100 IU/ml (prepare 2x 2,5 ml)
1	Vomit bowl (for single use) as disposal

b) Performance - step by step

1. Disinfection of the work surface
2. Set up materials (see material list)
3. Hand disinfection
4. Open sterile compresses
5. Open 5 ml syringe package (for blood collection)
6. Prepare 2x 2.5 ml syringe with Medusanal Heparin
7. Prepare 10 ml syringes PosiFlush XS
8. Thoroughly spray a pack of compresses with Octenisept
9. Inform and position the patient
10. Put on non-sterile gloves
11. Remove protective compresses
12. Dispose of gloves
13. Hand disinfection
14. Put on non-sterile gloves
15. Place sterile compress under the Broviac
    Note: Make sure that the terminals are closed.
16. Remove IN plug
17. Disinfection/cleaning of the connections with Octenisept compress
18. Change of gloves
    Note: The terminal must always be closed, the stopper must be in place or the syringe must be connected.
    For all following steps only use light pressure.
19. Carefully aspirate and discard 5 ml of blood at the thigh
20. Blood sampling
21. Rinse the Broviac with PosiFlush 10 ml XS
22. Block with 2.5 ml Medusanal Heparin 100 IU/ml

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23. Close leg with new IN plug.
    Note: Make sure that the clamps are closed.
    (If necessary, repeat this procedure for the second Broviac leg.)
24. Repack the leg with sterile compresses, if necessary fix with plaster/crocodile clip
25. Dispose of material
26. Documentation

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III. Blood and saliva sampling in WP5 Biology

The purpose of this Standard Operating Procedure (SOP) related to Biology WP is to ensure the procedure of blood sampling for patients included in this WP.

This SOP is specific to WP5 and do not include the procedures of blood sampling for the specific markers in WP2 (ex. BNP, NT-Pro-BNP, IGF-1, GH, LH etc). SOPs for WP2 tasks are available below describing the procedures for specific markers of interest in each task.

If there is any question or information needed, please contact:

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- SOP I – Participant Study ID
- SOP II – Blood sampling

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RADIOTHERAPY WP2	INTERVENTIONAL CARDIOLOGY WP3
Inclusion criteria	
• Age at diagnosis =< 21 years • Informed consent of parent/guardian as well as child/patient • Patients treated for : **brain tumours (except malignant gliomas);** **head and neck tumours (e.g. rhabdomyosarcomas and nasopharyngeal carcinoma); Hodgkin's lymphoma** • Patients receiving pulmonary and chest radiation for: **Ewing sarcoma; other chest sarcomas;** **Lung metastasis of Wilms and Ewing tumours, and other tumours** • Patients receiving craniospinal radiation therapy for: **Medulloblastoma or other tumours**	• Age of patients: 5-22 years • Patients with congenital heart disease • Informed consent of parent/guardian as well as child/patient
Exclusion criteria	
Chromosomal abnormalities and/or genetic syndromes Absence of informed consent	

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Inclusion
B0
Before irradiation

M0
After irradiation, up to 3 months

M12
1 year after irradiation

Each time 3 tubes with blood and 1 tube with saliva to be

BD Vacutainer CPT Mononuclear Cell Preparation Tube - Sodium Citrate) (4 ml)

Standard plastic tube without any preservative

1 EDTA tube (~3-5 ml)

Serum tube without anti-coagulant (~3-5 ml)

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As shown above, blood and saliva samples will be collected at 3 time points:

B0: Baseline ➔ Before start of radiotherapy (WP2)/interventional cardiology (WP3)

M0: End of radiation ➔ After radiotherapy up to three months (WP2) or day of finishing radiotherapy/interventional cardiology (WP3)

M12: ➔One year after finishing radiotherapy (WP2)/interventional cardiology (WP3)

7.1. Material needed for blood and saliva sampling

7.1.1. Tubes

Types of tubes	Number of tubes	Suggested reference
EDTA (3-5ml)	1 per patient per time	
CPT Tube - Sodium Citrate (4 ml)	1 per patient per time	https://www.bdbiosciences.com/us/applications/blood-collection/cell-biomarker-preservation/bd-vacutainerreg-cpttrade-mononuclear-cell-preparation-tube---sodium-citrate/p/362760
Clot activator serum separation tube (tube without anti-coagulant)	1 per patient per time	
Standard 10 or 15 ml plastic tube for collecting saliva	1 per patient per time	

7.1.2. Other materials

Type	Quantity needed	Suggested reference
Sterile 2 ml tubes with screw cap	Packages of 1000 tubes	https://www.sigmaaldrich.com/catalog/product/aldrich/br780763?lang=en&region=SE
Sterile Phosphate Buffered Saline, PBS	500 ml	https://www.sigmaaldrich.com/catalog/product/sigma/d8662?lang=en&region=S
Ice to keep sample at 0 to 4°C		

7.1.3. Equipment

Freezer (-80°C) and centrifuge adapted to different tubes

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7.2. Patient information and sample labelling (see SOP I)

Coding and labelling biological samples of each participating patient are done at each investigating centre after preparation of samples by a nurse or other dedicated medical staff.

Identifying data (full name, date of birth, hospital ID…) can be used for coding and labelling while biological samples/ DICOM file is carried out in the centre. However, all identifying data must be deleted and replaced by a unique participant identification number at HARMONIC registry level (**Participant Study ID**) by an authorized person at the centre before shipping the samples to the study Biobank/AQUILAB.

This ID will be created automatically after entering **Study Subject codes** and choosing **Inclusion: Country Centre** for the patient in HARMONIC REDCap database.

All collected samples are identified ONLY through **Participant Study ID**

Sample Information: Available samples Participant Study ID plus specify the type of biological material and the time points (B0; M0 and M12).

See detailed procedure and sample labelling in Annex III.1

7.3. Sample preparation and storage

At each time point, blood should be collected in 3 tubes as follows:

1. one vacutainer containing EDTA K2 (~ 4 ml),
2. one clot activator serum separation tube (a tube without anticoagulants) (~ 4 ml) and
3. in one BD Vacutainer® CPT™ tube for isolation of lymphocytes (~ 4 ml).

All blood tubes should be centrifuged within **2 hours of blood collection for best results**.

Steps by type of the tube

CPTblood tube* (to be analysed within 2 hours)	**Clot activator serum separation tube**	**EDTA tube**
Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times	Coagulation 30-40 min at RT	Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times
Blood centrifugation (1700 x g-force* 20 min at room temperature (RT))	Blood centrifugation (recommendation: 400 x g-force* 20 min at RT)	Blood centrifugation (recommendation: 400 x g-force 15 min at RT (19°C to 22°C)

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CPTblood tube* (to be analysed within 2 hours)	Clot activator serum separation tube	EDTA tube
		Seperate plasma and the rest of sample
Isolation of PBMC cell pellet	Serum (aliquots of ~ 1 ml) in 2-3 small tubes**	Save the plasma (3-4 aliquots of ~ 0.5 ml) preferable at 4°C
Washing cell pellet (lymphocytes) Discard supernatant without disturbing PBMC cell pellet (lymphocytes)	**Store part of the clot in 1 small tube** at -80°C**	Keep the rest of the blood (blood cells) in 2 aliquots in small tubes**
Please store all the materials (Lymphocytes; serum; clot, plasma; blood cells) in sterile 2 ml tubes with screw cap		
Store the dry pellet cells (lymphocytes) at -80°C	**Store both of them at -80°C**	**Store both of them at -80°C**

*Check detailed protocol for conversion of g-force to RPM in the Annex III.1
** Sterile 2 ml tubes with screw cap
Label each tube (see Annex III.2)
RT: room temperature

Before saliva sampling, it is important to require that patients do not eat 30 minutes before giving a saliva sample. Prior to saliva sampling, patients have to wash the mouth or drink water.

Collect approximately 4-5 ml saliva in a standard clean plastic tubes (10 or 15 ml adapted to the centrifuge) without any preservatives and divide the saliva in to the 2 or 3 small tubes with screw cap, almost 2 ml in each. The samples have to be transferred to -20r within 5-10 minutes.

7.4. Samples packaging and transfer

Each tube of cell pellet (lymphocytes), plasma, blood cells, serum, clot, saliva had to be labelled (Annex III.2). All samples will be sent from hospitals to Sweden SU, in boxes containing **dried ice** to keep the samples 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 Europe for analyses. The samples should be sent to SU, Sweden 2 times during the project: before month 36 (May 2022) and before month 49 (June 2023).

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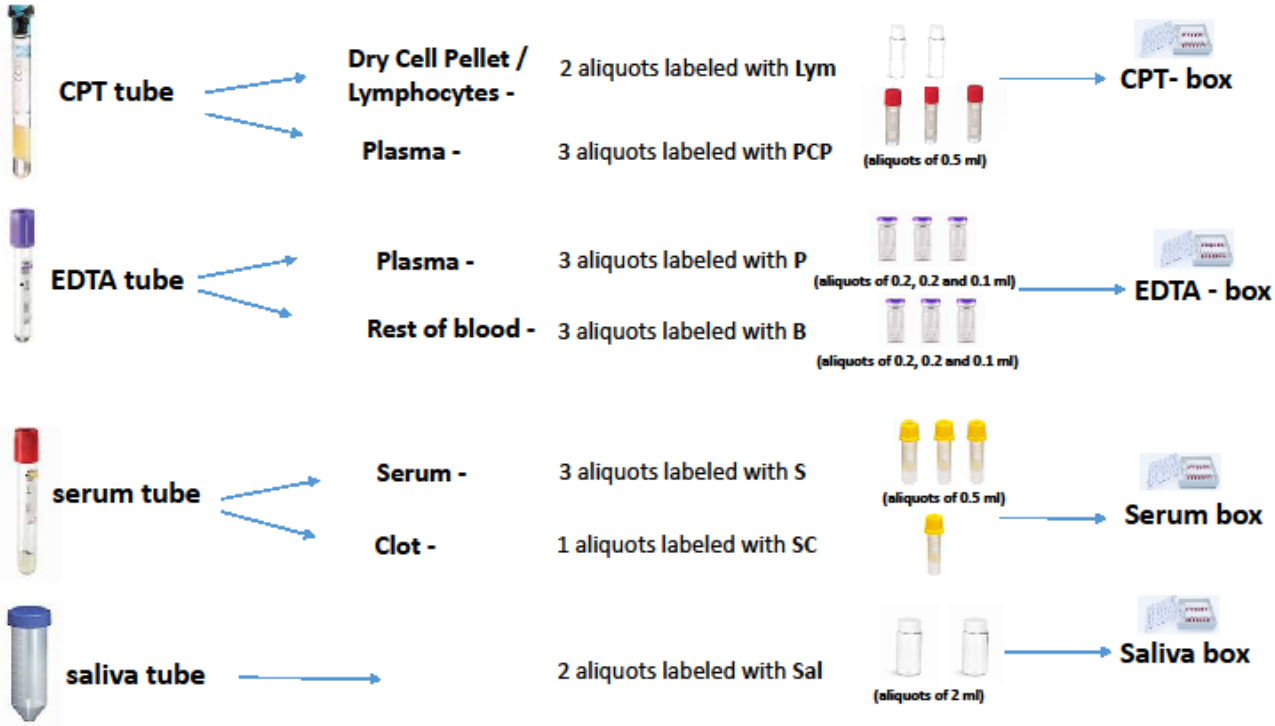
Annex III.1: detailed procedure and sample labelling



Each aliquot will be labeled with:
PATIENT ID + the **time point** (**B0** = Before IR, **M0** = After IR, **M12** = 1 year after IR) + the **type of biological materials** (**Lym**=Cell pellet/Lymphocytes **PCP**=Plasma from CPT, **P**= Plasma from EDTA, **B**= Rest of blood, **S**= Serum, **SC** =blood Clot, **Sal** = Saliva)

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Annex III.2: detailed protocol of Isolation of PBMC (Lymphocytes) using Cell Preparation Tubes (CPT)

This link could be helpful: www.youtube.com/watch?v=5Z25H8JLtDk

The BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate (CPT) is a single tube system for the collection of whole blood and the separation of mononuclear cells. Isolation of PBMC in these tubes occurred according to the manufacturer's instructions:

Steps:

1. Collect blood into CPT using venipuncture technique. Note: Blood tubes should be centrifuged within **2 hours of blood collection for best results**.

2. Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times.

3. Centrifuge CPT tubes at 1700 × g-force for 20 min *(PS: you should convert g-force (or also called RCF) to RPM for your particular centrifuge, please check provided link for coverting g-force to RPM)** at room temperature. Note: Do not centrifuge CPT over 2000 g-force, as it may cause tube breakage.

4. After centrifugation, carefully open the CPT in a biological safety cabinet II. Using a pipette (ex. Pasteur pipette), gently collect the mononuclear cells, which can be found in the layer just under the plasma.

5. Transfer cells to a 10 mL (or 15 mL) conical standard tube. Avoid vigorous pipetting that would disintegrate the gel plug itself.

6. Add 3 mL PBS (Dulbecco's Phosphate Buffered Saline) to wash cells. Mix cells by inverting tube 3 to 5 times.

7. Centrifuge at 400 × g-force for 8 min. Discard supernatant without disturbing cell pellet.

8. Resuspend cell pellet by gently tapping tube with index finger.

9. Add 3 ml PBS again and mix cells by inverting tube 3 to 5 times.

10. Split the volume in to 2 small tubes (sterile 2 ml tubes with screw cap)

11. Centrifuge at 400 × g-force for 5 min. Discard supernatant without disturbing cell pellet.

12. The dry PBMC pellet cells should be stored at -80ºC.

Store samples at -80ºC until transfer to the SU in dry ice

*For coverting g-force to RPM:https://www.sigmaaldrich.com/technical-documents/articles/biology/g-force-calculator.html

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IV. Blood sampling and anthropometry in Task 2.2 - Endocrine Dysfunctions

The purpose of this Standard Operating Procedure (SOP) is to ensure the procedure of blood sampling (provided that the collection is carried out at the participating partner centre) and anthropometry to obtain endocrine parameters under standardized conditions within the HARMONIC-RT study. It is valid for the blood collection and measurement of the first included patient before and after external beam radiotherapy (EBRT) and loses its validity after the last included patient whose EBRT has been completed (see WP2 protocol for inclusion and exclusion criteria).

2. Notes and remarks

After the revision of DIN EN ISO 9001: 2015, process orientation is a central concern of the standard, which is also to be found in the documentation. In this context, the interaction of the processes, i.e. the representation of the sequence of the individual activity steps, is more important than before.

These focal points in the SOP compiled here for you are to be adapted to the respective clinic and department. Superfluous items should be deleted and special items added. The abundance of information is a non-binding recommendation and should help to take into account all relevant areas and activities. Unless clinical routine in the centres conflicts with the principles of this SOP, blood draws may be performed according to standard procedures in the centres.

3.

- SOP I– Participant Study ID
- SOP II – Blood Sampling
- SOP III – WP5
- SOP IX– Dosimetry/Data transfer Aquilab/WP2 centres
- REDCap data entry guidelines (to be provided)
- eCRF – Endocrine dysfunction (BL and FU)

4.

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5. Inclusion and exclusion criteria

In addition to the general inclusion and exclusion criteria (WP2 protocol), clinical factors must be taken into account which justify the collection of hormone parameters (see Annex IV.1) and anthropometry (see chapter 7 of SOP IV and Annex IV.2) as a part of the routine. Hence, patients to be included are those facing an irradiation in the head and neck area (including the suprasellar region) at baseline before reaching adulthood (<18 years) [1]. That means it is assumed that the endocrine organs will be affected by radiation exposure. Patients for whom it is not medically indicated to determine the required hormone parameters are therefore excluded.

It is intended that patients included for endocrine assessment at baseline will also be followed-up by the respective participating centres. It is not necessary to perform the blood collection at the participating centre. However, the participating centre should ensure that the defined parameters are available at the specified times and should also document them in the eCRF intended for this purpose.

6. Blood sampling within task 2.2

6.1. Purpose

Blood sampling within the scope of Task 2.2 Endocrine Dysfunction, summarized in Table IV.1, serves as laboratory chemical determination of endocrine parameters, the results of which will be incorporated into HARMONIC's clinical database and contribute to answering scientific questions.

Table IV.1 Type and purpose of use

Institute	Laboratory	Sample (mandatory)	Sample (optional)
University Hospital Essen	Central Laboratory, University Hospital Essen	IGF-1, GH, LH, FSH, TSH, fT3 fT4, Estrogen, Testosterone, Progesteron,	ACTH, Prolactin
Gustave Roussy		IGF-1, GH, LH, FSH, TSH, fT3 fT4, Estrogen, Testosterone, Progesteron,	ACTH, Prolactin
DCPT, Aarhus University Hospital	Blood samples are taken at the local paediatric clinic.	IGF-1, GH, LH, FSH, TSH, fT3 fT4, Estrogen, Testosterone, Progesteron,	ACTH, Prolactin
KU Leuven		IGF-1, GH, LH, FSH, TSH, fT3 fT4, Estrogen, Testosterone, Progesteron,	ACTH, Prolactin

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7. Anthropometry

7.1. Requirements

7.1.1. Supplies

— **Devices/Inventory**

Amount	Equipment
1	Roller stool (for the examiner)
1	Metric Stadiometer
1	Measuring tape (in cm); suitable for cleaning
1	Surface disinfection (devices, work surfaces)
1	Pack of non-sterile gloves (different sizes)
1	Package garbage bags
1	Pack of tissues
1	Hospital bed/Couch (for very small patients)

7.1.2. Information

— **Patient Information**

- Local patient ID of medical record has to be matched with the name of the actual patient, before processing, in the direct interview of the patient.

7.2. Purpose, Preparation, Performance

7.2.1. Purpose

The evaluation of sitting height, abdominal girth and hip size in children and adolescence (<18 yr.) with head and neck tumors should provide additional information (besides body height and weight) on the functionality of the endocrine glands, with focus on the thyroid, hypothalamus and pituitary glade during and after radiotherapy.

7.2.2. Preparation

Measuring devices used on more than one patient pose a risk of infection and should be cleaned after use in accordance with local guidelines. Patients who have an infection but meet the inclusion criteria will also need to have their sitting height, abdominal girth and hip size measured; local infection and prevention measures should be used as precautions in these circumstances.

Non-sterile gloves are not routinely required for this procedure. The examiner must assess each patient for the risk of exposure to blood and body fluids [7] (Royal College of Nursing, 2018) and be familiar with local guidelines for glove use.

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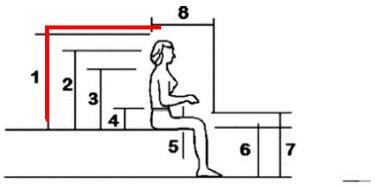
7.2.3. Performance

Please check the patient's identity on the basis of the patient file. Explain the procedure to the patient and allow time to ask questions. Then obtain verbal informed consent. Evaluate the patient's mobility and ability to stand unaided, then choose the appropriate method for measurement. Make sure that the equipment has been cleaned and decontaminate your hands according to local guidelines. An apron should be worn in case the patient needs physical help to get up from bed or chair.

a) Sitting height

The sitting height is measured from the crown of the head to the seat of the patient (see figure 1). The patient sits in an upright position on a flat stool with his/her spine facing the stadiometer. Curving of the back should be avoided as far as possible. Ask the patient to take a deep breath during the measurement and hold it for a short moment.



1	Sitting Height
2	Eye Height
3	Shoulder Height
4	Elbow Rest Height

Figure IV.1 Measurement of sitting height

For very young patients the crown-rump length is determined. Use a measuring tape for this. Place the infant on the side of a couch/hospital bed and start measuring from the crown to the tailbone. Please make sure that the small patient is as straight as possible. If necessary, you can ask a parent or assistant for help [8] [4]. Document patient's sitting height to the exact cm, in the patient file.

b) Abdominal girth

The abdominal girth is measured with a measuring tape at umblical level. Please ask the patient (if possible) to uncover his or her abdomen and make sure that the patient is in a straight position (standing or sitting). Place the measuring tape at umbilical level and lead it horizontally around the belly (see figure 2, A). Also make sure that the measuring tape is straight and not too tight around the patient [5]. In patients <2 years of age it may be appropriate to include an assistant or parent. Document patient's abdominal girth to the exact cm, in the patient file.

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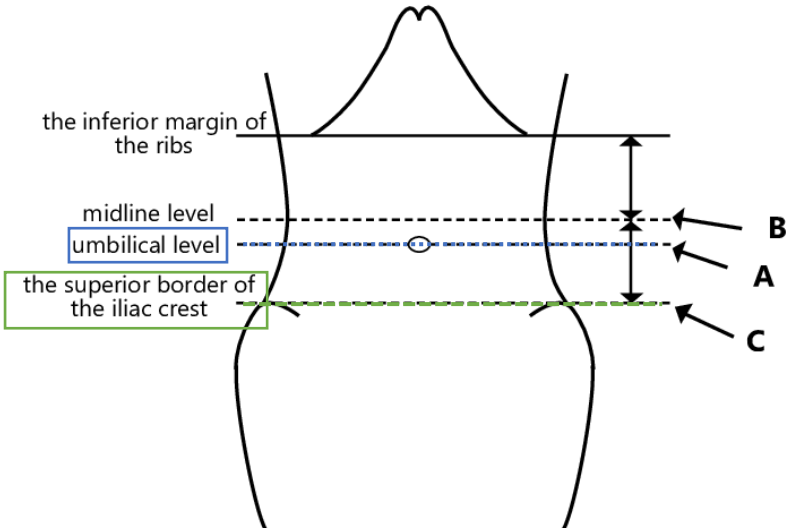

Figure IV.2 Measurement of abdominal girth and hip size

c) Hip size

For hip measurement, the measuring tape is placed at the level of the superior border of the iliac crest and is guided horizontally (not too tight) around the level (see figure 2, C). Please make sure that the measurement tape is guided as straight as possible to avoid incorrect measuring results. Document patient's hip size to the exact cm, in the patient file.

7.2.4. After Measurement

After taking the values, clean the workplace and accompany the patient to the next examination. Clean the measuring tape, work surface, metric stadiometer and if necessary, the hospital bed/couch with a surface disinfectant and disposable cloths. Thereby please pay attention to the local regulations. Throw away the used disposable cloths in the container assigned for this purpose.

Identification of patient

Unconfirmed patient identification can lead to missing traceability of the sample and to sample mix-ups. If there is any uncertainty about the subject or the sample, the sample should be discarded.

Local conditions and equipment

For blood collection, it must be checked whether clean and hygienic conditions are present. If there are any deviations, the sampling process must be stopped and the responsible person contacted in order to take corrective action and release the process again.

Storage and transport conditions

Storage and transport conditions must be controlled to ensure clean and hygienic environmental conditions for products, test samples and labels. In case of deviations, sampling

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is interrupted and the responsible person must decide on corrective measures as well as a subsequent release of the process.

Project Coordination and Management

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Annex IV.1: Blood Sampling

a. Parameters and Units

Parameter	Unit
IGF1	ng/ml
GH	ng/ml
ACTH	pg/ml
LH	IU/l
FSH	IU/l
TSH	mU/l
fT3	pmol/l
fT4	pmol/l
Estrogen	pg/ml
Testosterone	nmol/l
Progesteron	ng/ml
Prolactin	(ng/ml)

Please note: The determination of sex hormones in routine is usually carried out in patients in whom pubertal development disorders are suspected or have been diagnosed.

b. Time Frame of Collection

The following table shows the period of the parameters to be collected. The table also shows for each parameter the level of importance.

	Time points		
Parameter	**Baseline***	**M0****	**FU** (M24, M48, M120)
IGF1	M***	M***	O
GH	M***	M***	O
ACTH	O	O	O
LH	M***	M***	O
FSH	M***	M***	O
TSH	M***	M***	O
fT3	M***	M***	O

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Parameter	Time points		
	Baseline*	M0**	FU (M24, M48, M120)
fT4	M***	M***	O
Estrogen	M***	M***	O
Testosterone	M***	M***	O
Progesteron	M***	M***	O
Prolactin	O	O	O

Level of importance: M = mandatory, O = optional; *Baseline: At EBRT1 planning, or 2 weeks before or 1 week after EBRT 1st fraction, M0**: 90d; ***Mandatory in prospective data inclusion, optional for retrospective data collection. In the case that patients receive more than one blood test to assess hormone levels in the blood during the defined baseline period, the results of the test nearest to the first day of irradiation should be reported.

Annex IV 2: Anthropometry

Time Frame of Measurements

Parameter	Baseline*	End of EBRT	FU (M24, M48, M120)
Current sitting height (cm)	M**	------	O
Current abdominal girth (cm)	M**	------	O
Current hip size (cm)	M**	------	O

Level of importance: M = mandatory, O = optional; *Baseline: At EBRT1 planning, or 2 weeks before or 1 week after EBRT 1st fraction; ------ = no collection required, **Mandatory in prospective data inclusion, optional for retrospective data collection.

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Annex IV 3: Contouring guidelines for hypothalamus and pituitary gland

SEPARATE PDF OF GUIDELINES CAN BE PROVIDED ON DEMAND

Contouring guidelines for hypothalamus and pituitary gland in the HARMONIC project

- ***Purpose:*** Hypothalamus is a 2-4 cm^3 polygonal structure, consisting of two separate portions on each side of the third ventricle and is known to have functions such as homeostatsis (e. g. water and electrolyte balance, regulation of temperature and circadian rhythms), endocrine feedback (growth hormones, reproductive hormones etc.), control of autonomic nervous system and limbic functions (memory and emotion). Pituitary gland is attached to hypothalamus and is responsible for releasing of hormones that influence most endocrine systems in the body. In order to predict hormone deficiency after radiation treatment to hypothalamic-pituitary axis in the childhood, the impact of radiation burden to hypothalamus and pituitary gland is going to be investigated in the HARMONIC project.

Contouring of hypothalamus and pituitary gland

- ***Method:*** The planning CT and MRI of head should be performed in 1-2 mm slice thickness to outline the hypothalamic-pituitary substructures precisely. The definition is based on T1 weighted unenhanced MRI after exact fusion with the planning CT. Hypothalamus and pituitary gland, divided into 5 components, are mandatory to be contoured in the HARMONIC project:

1. anterior hypothalamus

2. posterior hypothalamus

3. infundibulum

4. anterior pituitary

5. posterior pituitary

The instructions described as follows serve as reference for the delineation.

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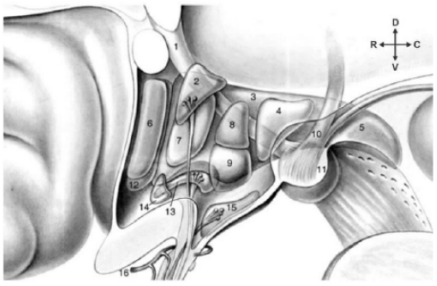
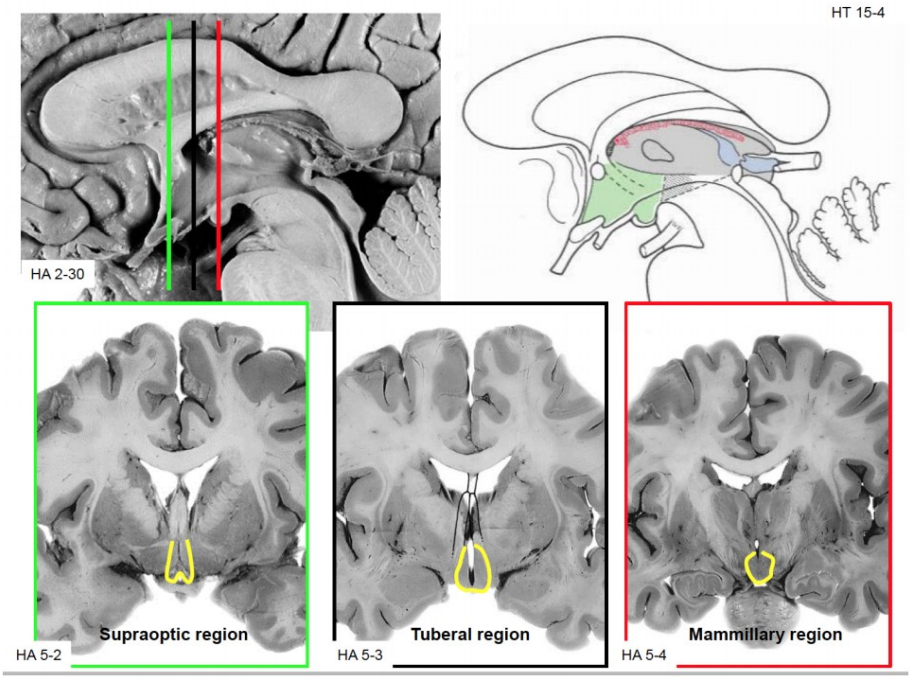
Parcellation of hypothalamus

Hypothalamus



Region	Ventral	Lateral	
preoptic part	Medial Preoptic Nucleus	Lateral Preoptic Nucleus	anterior hypothalamus
	Suprachiasmatic Nucleus → just above the optic chiasm, bordering the optic recess of the 3rd ventricle		
anterior part		Paraventricular Nucleus → overlapping with the fornix, hypothalamo-hypophyseal tract	
	Anterior Hypothalamic Nucleus		
		Supraoptic Nucleus	
		Infundibular/Arcuate Nucleus	
tuberal part	Dorsomedial Nucleus	Paraventricular Nucleus (superior-posterior edge)	
	Ventromedial Nucleus	Lateral Tuberal Nucleus	
	Infundibular/Arcuate Nucleus	Tuberomammillary Nucleus	
posterior part	Posterior Hypothalamic Nucleus		posterior hypothalamus
	Ventral Mammillary Nucleus	Lateral Mammillary Nucleus	

Anatomy of hypothalamus in situ



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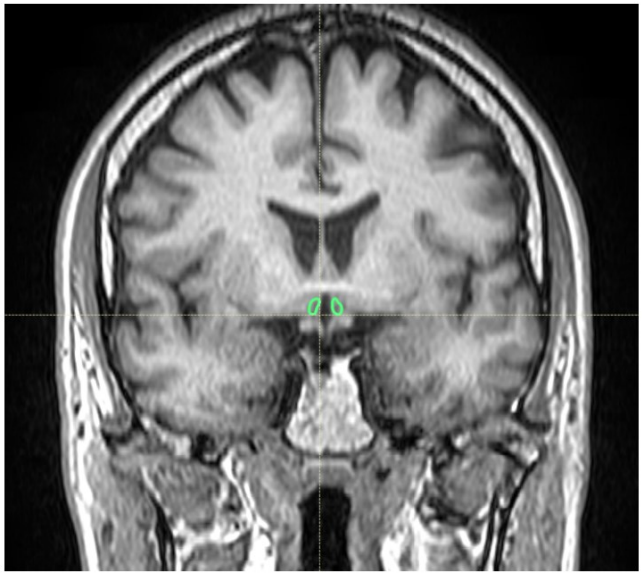
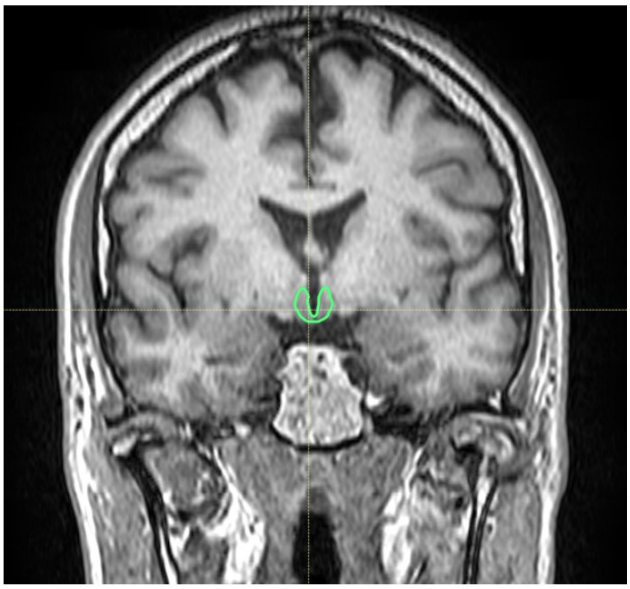
Step by step outlining of anterior hypothalamus: preoptic part



Anatomical boundaries of preoptic part:

Inferior: optic chiasm
Superior: middle of anterior commisure
Medial: third ventricle
Lateral: substantia innominata

Step by step outlining of anterior hypothalamus: anterior part



Anatomical boundaries of anterior part:

Inferior: optic tracts
Superior: columns of fornix
Medial: third ventricle
Lateral: internal globus pallidus

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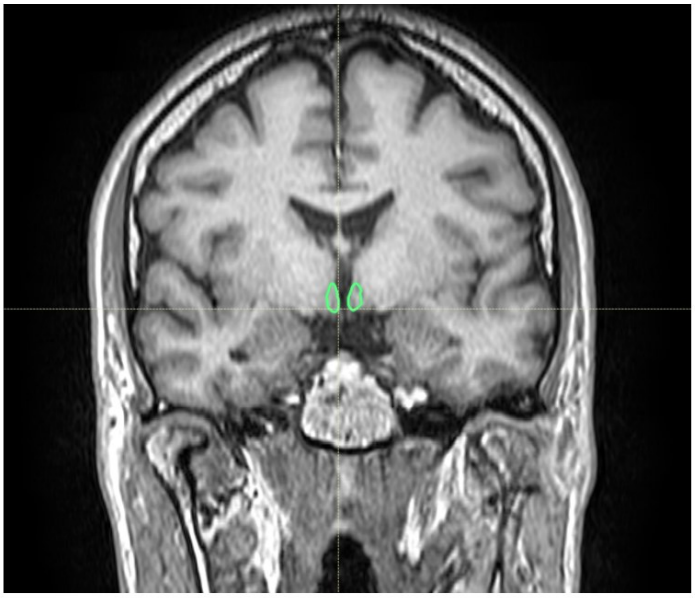
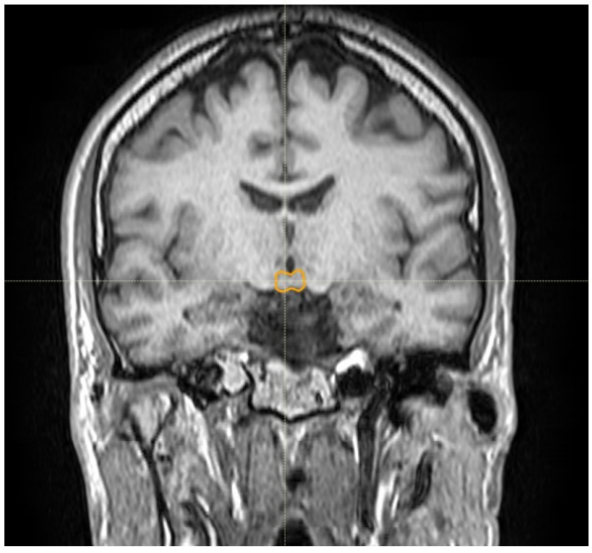
Step by step outlining of anterior hypothalamus: tuberal part



Anatomical boundaries of tuberal part:

Inferior: infundibulum
Superior: medial pole of internal capsule
Medial: third ventricle
Lateral: globus pallidus

Outlining of posterior hypothalamus



Posterior part:

including all slices of mammillary bodies

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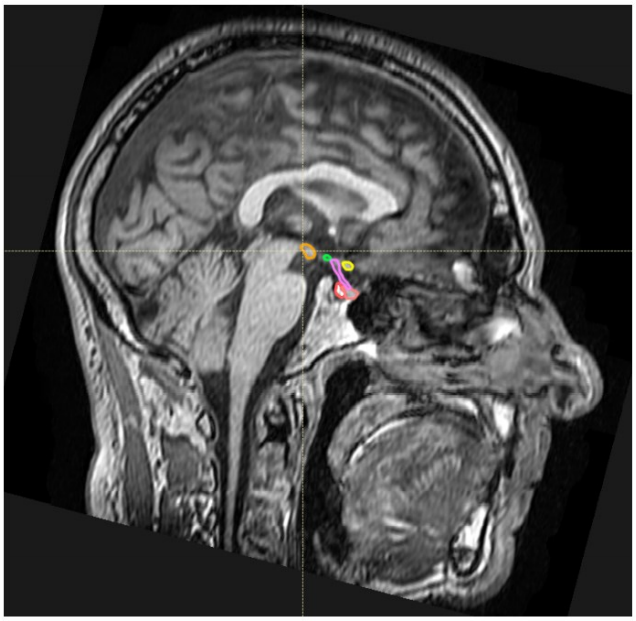
Pituitary gland and infundibulum



Pituitary gland is an oval structure lying in the sella turcica and is connected with hypothalamus by the stalk (infundibulum). It is laterally bordered by the cavernous sinuses and can be best defined in the sagittal view.

Anterior hypothalamus

Posterior hypothalamus

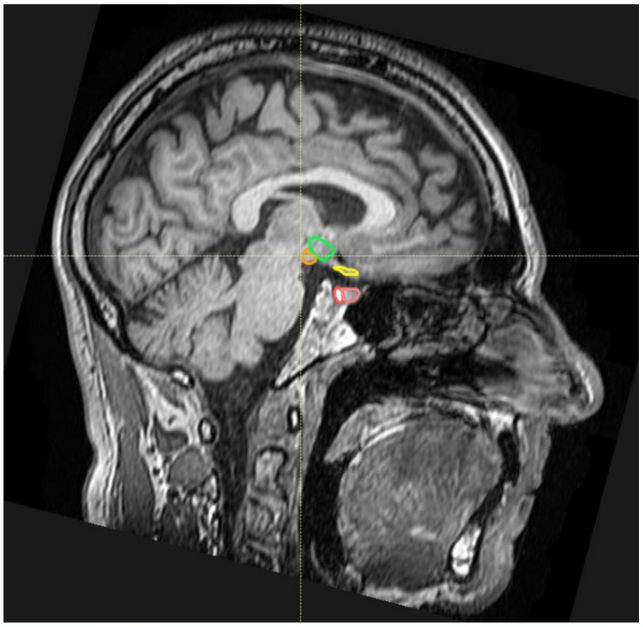
Optic chiasm

Infundibulum

Anterior pituitary

Posterior pituitary

Pituitary gland and infundibulum



The anterior and posterior portions of pituitary gland can be simply differentiated in T1 weighted sequence, since the posterior pituitary has intrinsic high T1 signal and presents itself as a bright spot.

Anterior hypothalamus

Posterior hypothalamus

Optic chiasm

Anterior pituitary

Posterior pituitary

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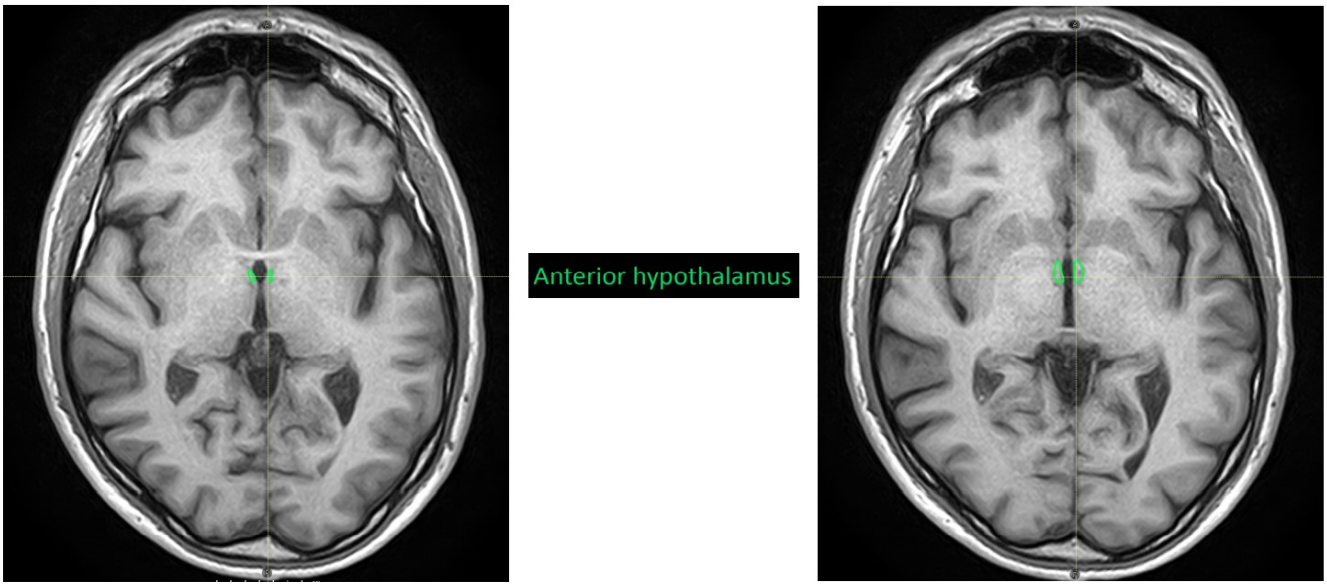
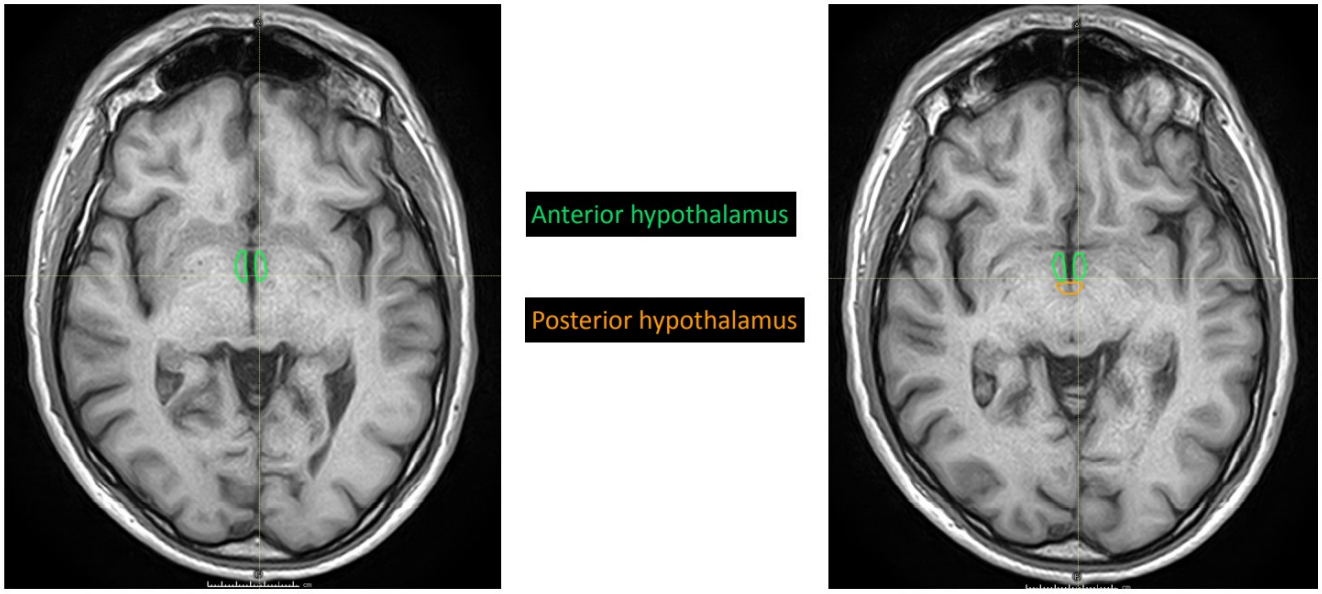
Axial presentation of hypothalamic-pituitary substructures



Axial presentation of hypothalamic-pituitary substructures



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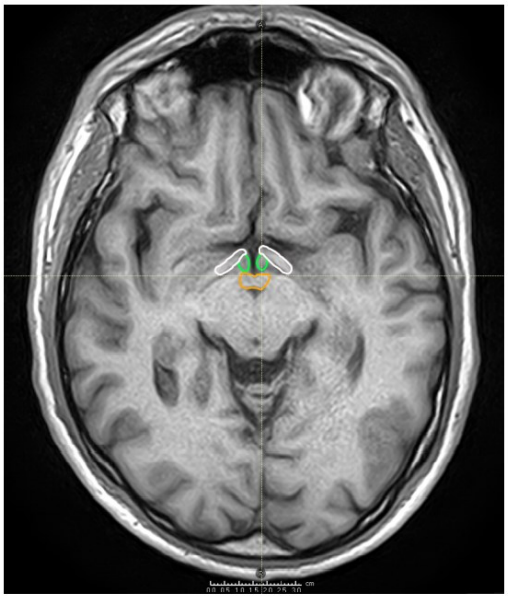
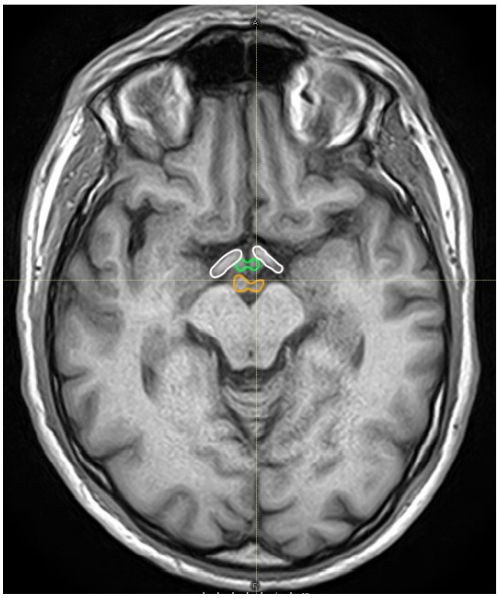
Axial presentation of hypothalamic-pituitary substructures



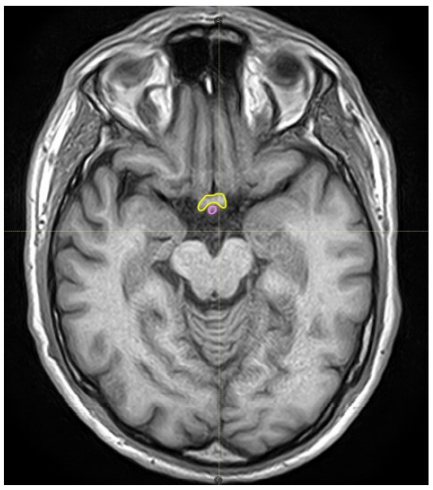
Optic tract

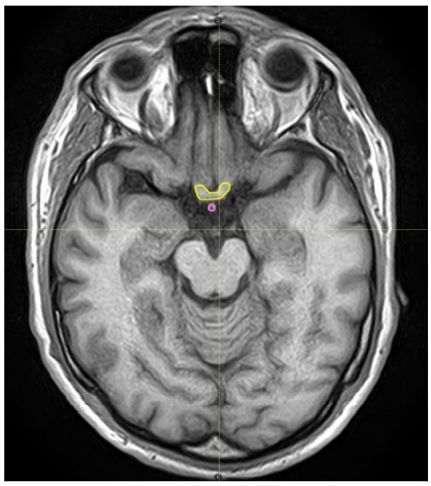
Anterior hypothalamus

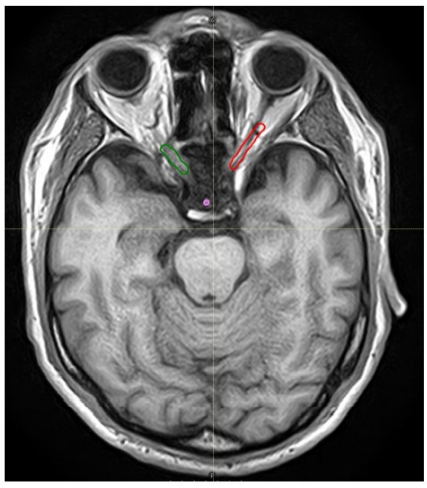
Posterior hypothalamus



Axial presentation of hypothalamic-pituitary substructures







Optic chiasm

Infundibulum

Right optic nerv

Left optic nerv

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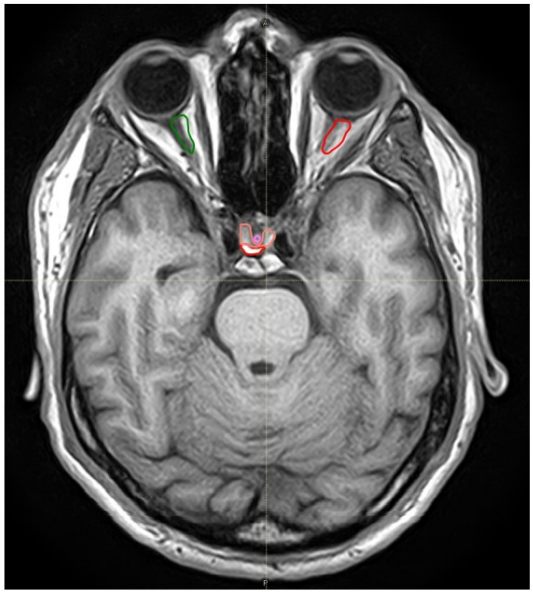
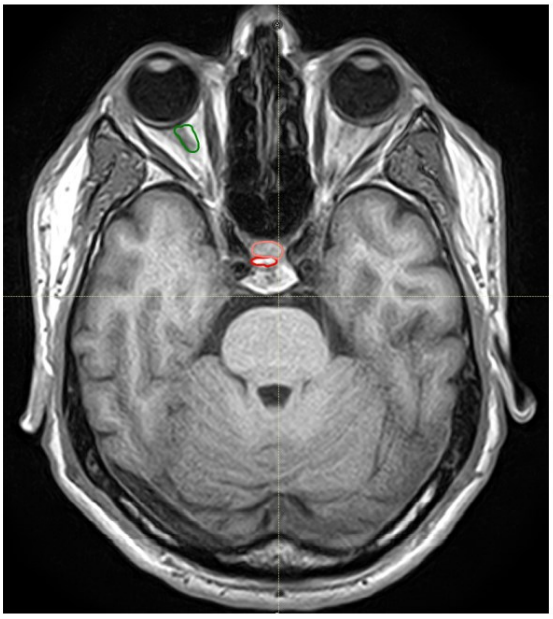
Axial presentation of hypothalamic-pituitary substructures



Anterior pituitary

Infundibulum

Posterior pituitary



This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No. 847707

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V. Blood sampling and cardiac parameters in Task 2.3 - Cardiovascular toxicities

The purpose of this Standard Operating Procedure (SOP) related to cardiovascular task (Task 2.3) is to ensure the procedure of imaging and biological biomarkers for patients included in this task.

If there is any question or information needed, please contact:

Responsible at Gustave Roussy (France)	Email
Dr. Stephanie BOLLE	Stephanie.bolle@gustaveroussy.fr
Dr. Brice FRESNEAU	Brice.fresneau@gustaveroussy.fr
Dr Nadia HADDY	Nadia.haddy@gustaveroussy.fr
Dr Maryline LEVY	
Dr Thichien TRAN	Thichien.TRAN@gustaveroussy.f

- SOP I – Participant Study ID
- SOP II – Blood Sampling
- eCRF – Cardiovascular toxicity
- HARMONIC guidelines for data entry in the clinical database (to be provided)

Partners	Clinicians & Epidemiologists
Gustave Roussy (France)	Dr. Brice Fresneau (pediatric oncologist) Dr. Stephanie Bolle (radiation therapist) Dr. Valentine Martin (radiation therapist) Dr Maryline Levy (cardiologist) Nadia Haddy (epidemiologist)
Clinic for Particle Therapy – University Hospital Essen (Germany)	Prof. Beate Timmermann
DCPT, Aarhus University Hospital (Denmark)	Dr. Yasmin Lassen Dr. Sonja Karabegovic
KU Leuven (Belgium)	Dr. Gilles Defraene Dr. Karin Haustermans

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- Patients treated for solid tumor (included central nervous system tumor) or lymphoma
- Diagnosed at an age <22 years-old
- Treated with external beam radiation therapy (EBMRT) as part of their oncological treatment
- With one or more of following EBRT fields:
  1. *Mediastinal irradiation*
  2. *Bipulmonary irradiation*
  3. *Chest radiation (including vertebral and paravertebral radiation) with at least one part of the heart in, or at the edge of, radiation field*
  4. *Craniospinal radiation*
  5. *Abdominal radiation with at least one part of the heart in, or at the edge of, radiation field*
- Informed consent of parent/guardian for patients aged <18years at inclusion
- Informed consent of patient if aged ≥18years at inclusion

Time points	
B0	Before RT
T1	After radiotherapy up to 3 months
T2	1 year after radiotherapy
T3	5 years after radiotherapy
Optional	10 years after radiotherapy and then every 5 years

7.1. Imaging parameters

7.1.1. Time Frame of Collection

	B0	T1 M3	T2 M12	T3 M60	Optional M120
Cardiac Echography Ejection Fraction, mode Simpson Biplan	X	X	X	X	X

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Ejection Fraction, mode Teicholz Shortness fraction Cardiac frequency Diastolic function (mitral E/A ratio) Left ventricular size (end-diastolic dimension z-scores) Pulmonary artery systolic pressure					

*M: months number

7.1.2. Requirements

Echocardiogram

7.1.3. Potential risks

No risk identified

7.1.4. Purpose

Early changes in imaging markers of cardiovascular damages (cardiac echography)

- ejection and shortness fraction
- others (e.g. cardiac frequency, mitral E/A ratio, end-diastolic dimension z-scores)

7.2. Biological parameters

7.2.1. Time Frame of Collection

Specific blood sampling for WP2.3 markers (EDTA tube). Markers measured at centres	B0	T1 M3	T2 M12	T3 M60	Optional M120
NT-proBNP	X	X	X	X	X
Troponine	X	X	X	X	X
CPK	X	X	X	X	X
Total cholesterol, HDL, LDL	X	X	X	X	X
Triglycerides	X	X	X	X	X
Glycosylated haemoglobin (HbA1c)	X	X	X	X	X
Hemoglobin	X	X	X	X	X
Creatinine	X	X	X	X	X
Ferritin	X	X	X	X	X

7.2.2. Requirements

See Table II.1 and Table II.2 in SOP II Blood sampling

Project Coordination and Management

FUNDACION PRIVADA INSTITUTO DE SALUD GLOBAL BARCELONA (ISGLOBAL), established in C ROSSELLO 132 PLANTA 05, BARCELONA 08036, Spain.

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Task 2.3 Co-Leaders

Stéphanie Bolle, Brice Fresneau, Nadia Haddy

GUSTAVE ROUSSY (GR), established in Rue Camille Desmoulins 39, VILLEJUIF 94805, France

Task 2.3 Partner

Centre Régional François Baclesse

KATHOLIEKE UNIVERSITEIT LEUVEN (KU Leuven), established in OUDE MARKT 13, LEUVEN 3000, Belgium

AARHUS UNIVERSITETSHOSPITAL (AUH), established in PALLE JUUL-JENSENS BOULEVARD 99, AARHUS 8200, Denmark

AARHUS UNIVERSITET (AU), established in NORDRE RINGGADE 1, AARHUS C 8000, Denmark

UNIVERSITAETSKLINIKUM ESSEN (UKESSEN), established in HUFELANDSTRASSE 55, ESSEN 45147, Germany

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VI. Neurovascular Toxicity Task 2.4

The purpose of this Standard Operating Procedure (SOP) is to provide more specific information and guidance on which data need to be collected related to WP2 task 2.4. Neurovascular. This way, harmonized data collection across all recruiting clinical centres will be guaranteed in order to ensure high quality data collection and analysis. The SOP also describes the workflow required for the task in detail.

Neurovascular late effects after radiotherapy for childhood brain tumors are not well described in the literature. The neurovascular damage following irradiation can be divided into small or large vessel disease.

MRI changes characteristic for small vessel diseases are microbleeds, lacunar infarcts and white matter hyperintensities. These image changes are similar to those seen in elderly non-irradiated patients and can be associated to neurocognitive decline. Another aspect of small vessel disease after radiotherapy is the formation of cavernomas, which is believed to be caused by neoangiogenesis after damage to the small vessels of the brain.

Long-term survivors of pediatric brain tumors are also at an increased risk of cerebrovascular accidents compared to the general population due to large vessel atheropathy. Damage to the central arteries at the base of the brain may lead to stenosis or occlusion of the intracranial vessels or lead to inflammation as seen in Moya Moya disease. Clinically, patients with large vessel disease may present with transient ischemic attacks, intracranial hemorrhages or ischemic strokes. The risk of developing a cerebrovascular accident is likely related to the radiation doses received in specific neurovascular structures.

1.1. General aim

We will investigate and validate prospectively the extent and risk factors related to neurovascular events, quantify radiation dose-volume relationships in neurovascular structures for the development of neurovascular pathologies, and explore imaging changes as a precursor for neurovascular events.

1.2. Specific aims

- The frequency and degree of clinical symptoms associated with neurovascular toxicity after radiotherapy with modern techniques will be evaluated. In patients also included in task 2.6. these symptoms will be set in relation to the quality of life, level of fatigue and academic achievement.
- Image changes corresponding to small and large vessel disease will be systematically recorded and set into relation with the development of clinical symptoms and for patients participating in WP5 also with vascular biomarkers. The Black Blood MRI sequence will be evaluated in a subgroup of patients to see whether it better can visualise inflammatory processes in large vessel walls than other sequences.
- An atlas of neurovascular structures for delineation has been developed for this study. The radiation dose to these structures as well as to the whole brain, the brainstem and neurovascular pathologies seen on MRI will be extracted and analysed to evaluate dose-volume effects.
- SOP I Participant Study ID

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- SOP III WP5
- SOP VIII Quality of Life
- SOP description of upload of DICOM files to the Aquilab platform WP4
- Guidelines on data entry in clinical database

COUNTRY	INVESTIGATING CENTRE	ROLE IN TASK 2.4	PRINCIPAL INVESTIGATOR
Denmark	AU - Danish Centre for Particle Therapy | AUH - Aarhus Universityhospital	Leader	Dr. Yasmin Lassen
Belgium	Department of Radiation Oncology, UZ Leuven	Partner	Prof Dr. Karin Haustermans
Germany	Universitätsklinikum Essen (AöR) Klinik für Partikeltherapie Westdeutsches Protonentherapiezentrum Essen (WPE) Partnerstandort im Deutschen Konsortium für Translationale Krebsforschung	Partner	Prof Dr. Beate Timmermann
France	CRFB - Centre François Baclesse, Service Radiothérapie	Partner	Prof Dr. Juliette Thariat
	Gustave Roussy (GR) Department of Radiation Oncology	Partner	Dr. Stéphanie Bolle

Inclusion Criteria

- Patient included in the prospective part of HARMONIC
- Tumour in the brain or base of skull
- Possibility of MRI with neurovascular sequences in follow up

Exclusion Criteria

- Diffuse Pontine Glioma, Glioblastoma
- Previous irradiation in the brain

This task will assess the associations between MRI imaging markers, radiation dose to neurovascular structures, neurological symptoms, quality of life, level of fatigue, academical development and vascular biomarkers to assess neurovascular toxicity after paediatric radiotherapy with modern radiotherapy techniques.

5.1. Acquisition of neurovascular MRIs

The neurovascular MRIs (**nvMRI**) used in this study will generally be MRIs taken in the clinical routine for tumour assessment. The sequences will therefore include the sequences for tumour assessment and specific neurovascular sequences to assess small and large vessel disease.

A **nvMRI** should therefore **at least** include the following sequences:

- T1 with and without contrast
- T2 or FLAIR
- DWI

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- SWI or T2 STAR
- MR Angio or TOF
- (Patients treated at AUH Black Blood)

The MRI sequences used in the study **will not be further harmonised**, but should be taken after the usual protocols used in the different centres.

For questions or more information concerning the MRI sequences, please contact
Ronni Mikkelsen (ronni.mikkelsen@rm.dk)
Sanja Karabegovic (sanjkara@rm.dk)

A **nvMRI** should be performed at the following timepoints:

- Baseline (simulation MRI or last diagnostic MRI before radiotherapy)
- **M12** (12 months after end of radiotherapy)
- **M36** (36 months after end of radiotherapy)
- **M60** (60 months after end of radiotherapy)
- M120 (120 months after end of radiotherapy)

The **nvMRIs** at 12 months, 36 months and 60 months after radiotherapy are **mandatory**.

The neurovascular sequences in the Baseline MRI are optional, if not available, the last diagnostic MRI or the simulation MRI before radiotherapy will be counted as the baseline MRI even if the neurovascular sequences are not included. This scan should be uploaded (see below) even if the neurovascular sequences are not included.

The M120 scan is optional, as patients often are not followed for tumour assessment after five years. We will evaluate at a later timepoint whether the protocol should be amended to include a M120 **nvMRI** or even repetitive later **nvMRIs**.

There is a certain time tolerance for acquisition of the **nvMRI**s so that they can be coordinated in the clinical routine of tumour assessment:

There is a **margin of +/- 3 months** to the individual timepoints:
nvMRI M12 at 1 year after RT: corresponds to 9-15 months after RT
nvMRI M36 at 3 years after RT: corresponds to 33-39 months after RT
nvMRI M60 at 5 years after RT: corresponds to 57-63 months after RT

The acquisition of the MRIs will need to be coordinated between the department following the patient after radiotherapy and the radiological department. The participating centres are responsible to coordinate this so that it is assured that the **nvMRI**s are acquired as needed for this study.

It is also necessary to coordinate at the participating centre level the pseudonymization of the **nvMRI**s and the MRI reports with the patient's unique HARMONIC participant ID and without any other patient identifiers. The participants ID will be known in the including centre (See SOP I). The pseudonymized MRIs will then be uploaded in DICOM format to the Aquilab database. (This procedure is not yet described as the Aquilab database is not yet functional in the HARMONIC study.) The scans should be uploaded latest three months after acquisition, earlier if possible, to permit an optimal workflow for the analysis at AUH. The date of the MRIs should be noted in the HARMONIC Redcap database and the pseudonymized reports of the MRIs

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should also be uploaded there (see for further detail in the annexe section). There is no need of translation of the reports.

If a centre is not using the Aquilab platform for the HARMONIC study, please contact Yasmin Lassen (yasmin.lassen@auh.rm.dk) to see how the MRI can be shared in that case.

The **nvMRI**s will be reviewed by the radiologists from AUH and imaging markers for large and small vessel disease will be recorded by them in the HARMONIC Redcap database.

5.2. Radiation dose plans

The clinical radiation dose plans will be collected after end of radiation therapy by uploading them pseudonymised with the patient's unique HARMONIC ID to the Aquilab platform. Neurovascular structures will be delineated in Aquilab by the AUH/AU team and dose volume information important for this task will be extracted and recorded by the team in the HARMONIC Redcap database. The radiation dose plans should be uploaded not later than 3 months after end of radiotherapy, earlier if possible.

If a centre is not using the Aquilab platform for the HARMONIC study, please contact Yasmin Lassen (yasmin.lassen@auh.rm.dk) to see how the radiation dose plans or information about them can be shared in that case.

5.3. Clinical information

The participating centre that includes and follows the patient needs to update the HARMONIC Redcap database regularly with the clinical information of the patient. This information should be added at baseline and at the timepoints M12, M36, M60 and if the protocol is amended also at M120 or potential later timepoints. The clinical information should be updated with latest 3 months after these timepoints, earlier if possible.

The clinical information will consist of information on the development of neurovascular events (like stroke, transitory ischemic attack (TIA), prolonged reversible ischemic deficit (PRIND) and haemorrhages seen by the clinician or on imaging) and the recording of specific neurological symptoms often seen in association with neurovascular disease that also could only be a precursor of an event. Furthermore, medication that could have an influence on neurovascular events, will be recorded regularly. At baseline predisposing syndromes like Neurofibromatosis type 1 (NF1) and a familial history of neurovascular events will be entered into the database.

In the annexe the recording of this information into the HARMONIC Redcap database is described in more detail.

5.4. Parameters collected in task 2.6. and WP5

It is not mandatory that patients that participate in task 2.4. also participate in task 2.6. and/or WP5. In case of their participation in these studies as well, we will make further investigations:

- As small vessel disease can have an impact on neurocognition, the neurovascular task will also include to study the association of the development of neurovascular toxicity and the patient's quality of life, level of fatigue and academic achievement, these parameters will be collected in the frame of the task 2.6. (see specific SOP VIII for that task).

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— Together with the radiobiology group in WP5, we will study the association of the development of neurovascular toxicity and the incidence of vascular biomarkers. Specific biomarkers are not yet defined, but a larger array of known vascular biomarkers will be investigated for potential associations. The biological material and the data will be collected in the frame of WP5 (see specific SOP III for WP5).

In the annexe the recording of this information into the HARMONIC Redcap database is described in more detail.

5.5. Timeline for collection of the studied parameters

	B0	M0	M12	M36	M60	M120	>M120
• nvMRI	X		X	X	X	*	*
• radiation dose plan		X					
• clinical data	X		X	X	X	*	*
• vascular biomarkers	X	X	X	*	*	*	*

Table VI.1: Timetable for measurements and collection of the studied parameters,
* if the protocol is amended to include more data collection at these timepoints

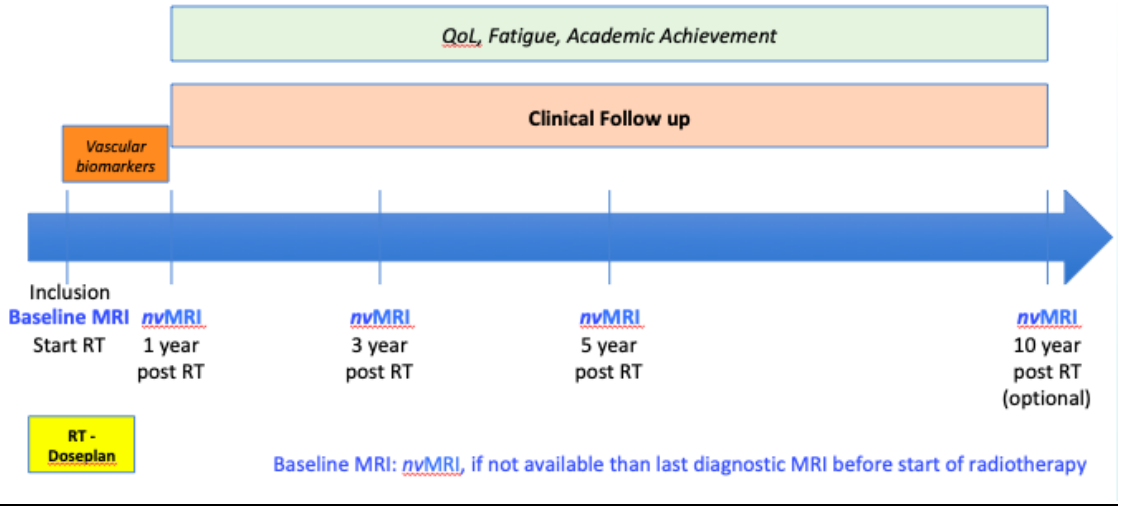

Figure VI.1: timeline of the data collection, the protocol might be amended to permit longer data collection, at baseline if nvMRI either the simulation MRI or the last diagnostic MRI should be uploaded

6.1. At patient inclusion

The participating centres include patients into the WP2 task 2.4. according to their internal procedure.

When a patient is included, please inform our research nurse Dorte Winther (dorwin@rm.dk) that a patient has been included into the task and indicate in the HARMONIC Redcap database that the patient is included into task 2.4.

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6.2. Uploading of data to the Aquilab database and completion of the eCRF for the neurovascular task by the participating centres

To assure data protection, all DICOM data, that are uploaded to the Aquilab platform will be pseudonymised by the participating centres with the unique participant's study ID that is known to the including centres (see SOP I Participant Study ID). This is also valid for the MRI reports that will be uploaded to the eCRF. No other patient identifier may be visible on these data.

The uploading procedure of DICOM data is not yet available as the Aquilab platform is not yet implemented into HARMONIC, the SOP will be updated once this information is available.

The completion of the eCRF is described in detail step by step in the Annexe.

6.3. Data collected for this study by the AUH/AU team concerning all patients included in the study

The AUH/AU team will do the following for all patients:

- review all baseline and nvMRI for neurovascular image markers (see annexe for list) and enter them into the eCRF
- delineate neurovascular structures on the CTs/MRIs in Aquilab and extract dose/volume parameters from the radiation dose plans and enter them into the eCRF

6.4. Dataflow

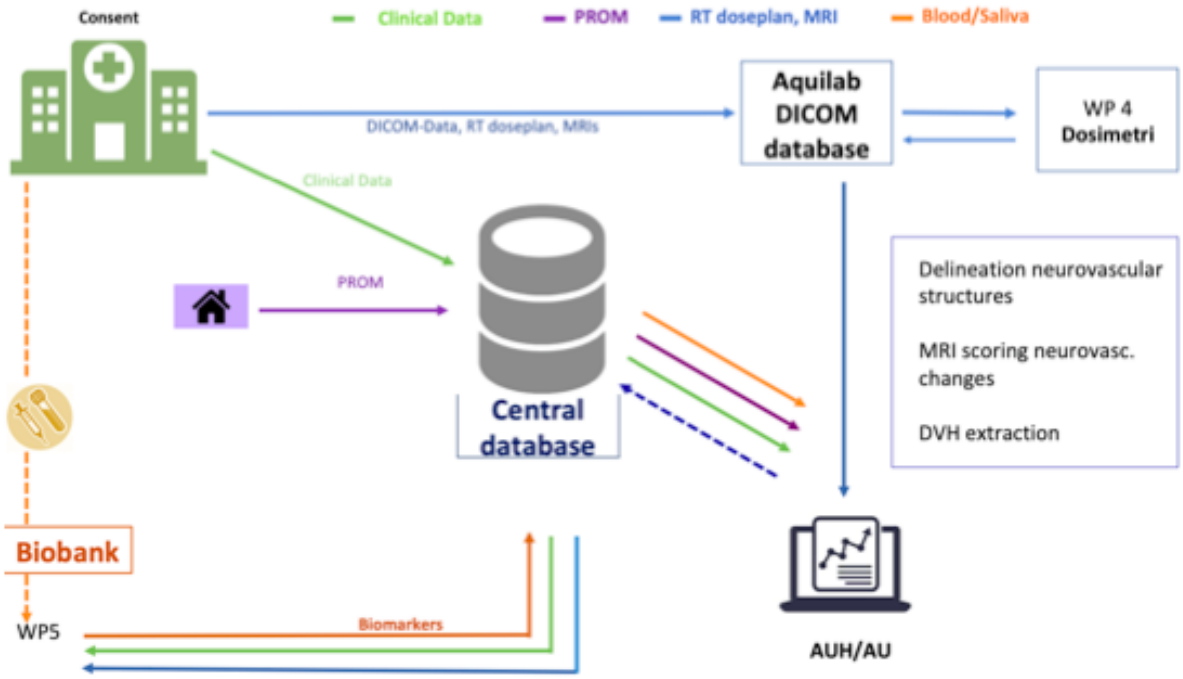

Figure VI. 2: Proposed dataflow for the neurovasular task, PROM stands for the data collected in task 2.6

For the final HARMONIC report in June 2024, we will correlate the early neurovascular clinical toxicity with imaging markers, the serological markers, dose to the neurovascular structures and QoL/fatigue/academic achievement data from task 2.6.

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We would like to organize a scientific conference on neurotoxicity after RT in 2024 to present our results and discuss the further research in the neurovascular task.

We will be elaborating together with all WP2 partners and WP5 the HARMONIC report on the neurovascular toxicity. Further reports on later toxicity are expected

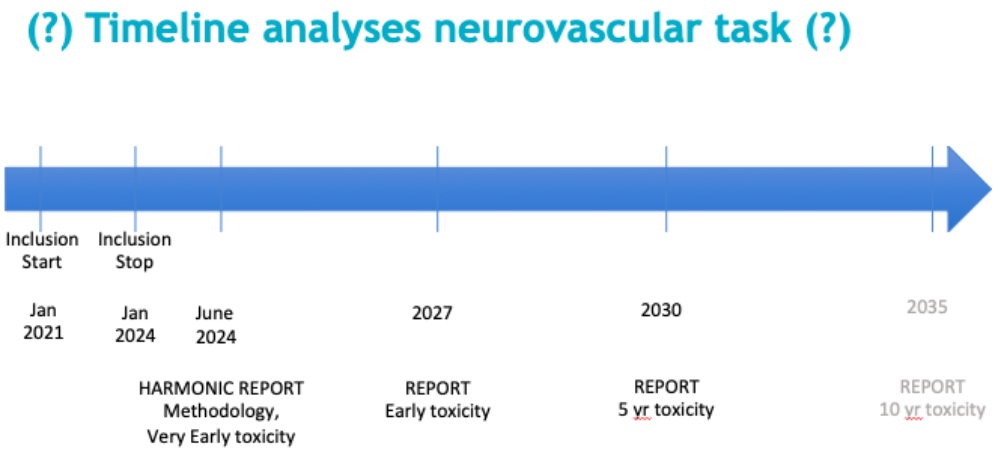

Figure VI.3: Approximate timeline of expected reporting from the neurovascular task

Any questions related to this SOP and the procedures described herein can be addressed to Yasmin Lassen, Danish Centre for Particle Therapy, AUH.
yasmin.lassen@auh.rm.dk
Tel.: + 45 51418065

A table with the contacts in the different centres associated to the neurovascular task can be found in the Annex VI.3.

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- **At inclusion**
  1. Contact Dorte Winther at dorwin@rm.dk and inform her about the inclusion of the patient
  2. Check the box **HARMONIC 2.4** in the patient ***Identification*** window

- **Neurovascular (nvMRI):** These must include at least all of the following sequences:

  - T1 with and without contrast
  - T2 or FLAIR,
  - DWI
  - SWI or T2 STAR
  - MR Angio or TOF

- **nvMRI is mandatory at M12, M36 and M60**
- a baseline MRI needs to be uploaded, if neurovascular sequences are not feasible for that, then upload the simulation MRI or the last diagnostic MRI
- the study can be amended in the future to permit further follow up with **nvMRIs**.
- for questions concerning the **nvMRI**s, please contact our neuroradiologists
  - Ronni Mikkelsen at ronni.mikkelsen@rm.dk or
  - Sanja Karabegovic at sanjkara@rm.dk

- **The DICOM data of the MRIs and the radiation dose plans and the reports of the MRIs need to be pseudonymized with the unique participant identification number at the HARMONIC registry level (*Participant Study ID*) by an authorized person at the centre before shipping to AQUILAB, no other identification is permitted.**

- The image markers and radiation dose-volume parameters will be extracted from the Aquilab platform by the team from AUH/AU and entered by them into the HARMONIC Redcap database.

- For further information or questions on the neurovascular task, please contact
  - Yasmin Lassen at yasmin.lassen@auh.rm.dk

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Annex VI.1:

Step by Step Guide for data entry into the eCRF in the central HARMONIC Redcap database relevant for the neurovascular task

1. Baseline_Patient inclusion into WP2 neurovascular task 2.4.

In the HARMONIC Redcap database baseline window, please find in the Identification column a row to define in which substudies a patient participates, tick the HARMONIC_2.4. box to clarify that the individual patient is participating in the neurovascular task.

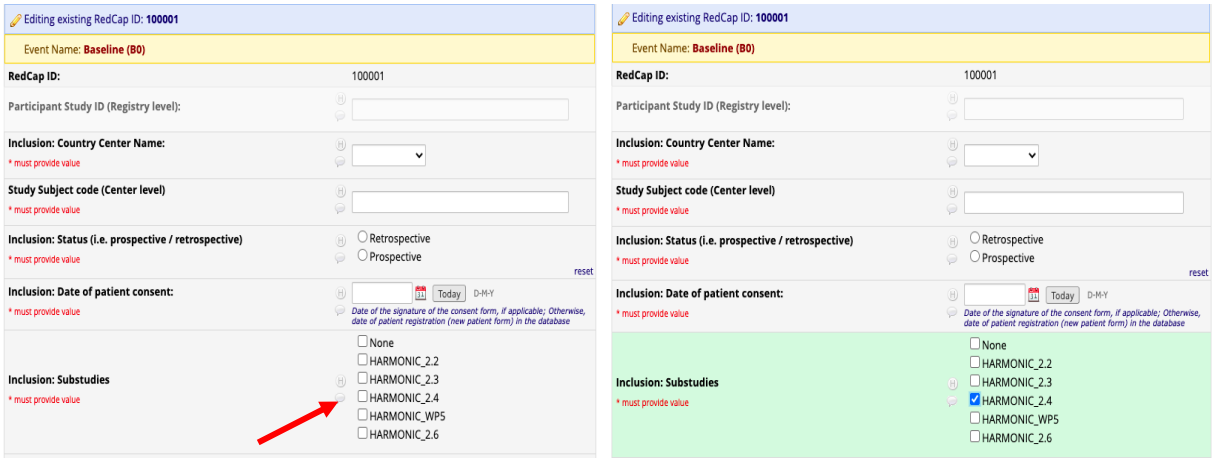

Figure VI. 4: Indication of patient inclusion in HARMONIC_2.4., neurovascular task

2. Baseline_Indication of predisposition for neurovascular diseases

In the HARMONIC Redcap database baseline window, please find the BL&FU-General Health Info window, to define whether a patient has a cancer predisposition syndrome (especially patients with NF1 have known neurovascular morbidities and there might be other genetical predispositions), or whether there is a family history for cerebrovascular accidents. Please note in the family history under others if there is in the patient's family a history of thrombosis, Factor V Leiden mutation or other coagulopathies.

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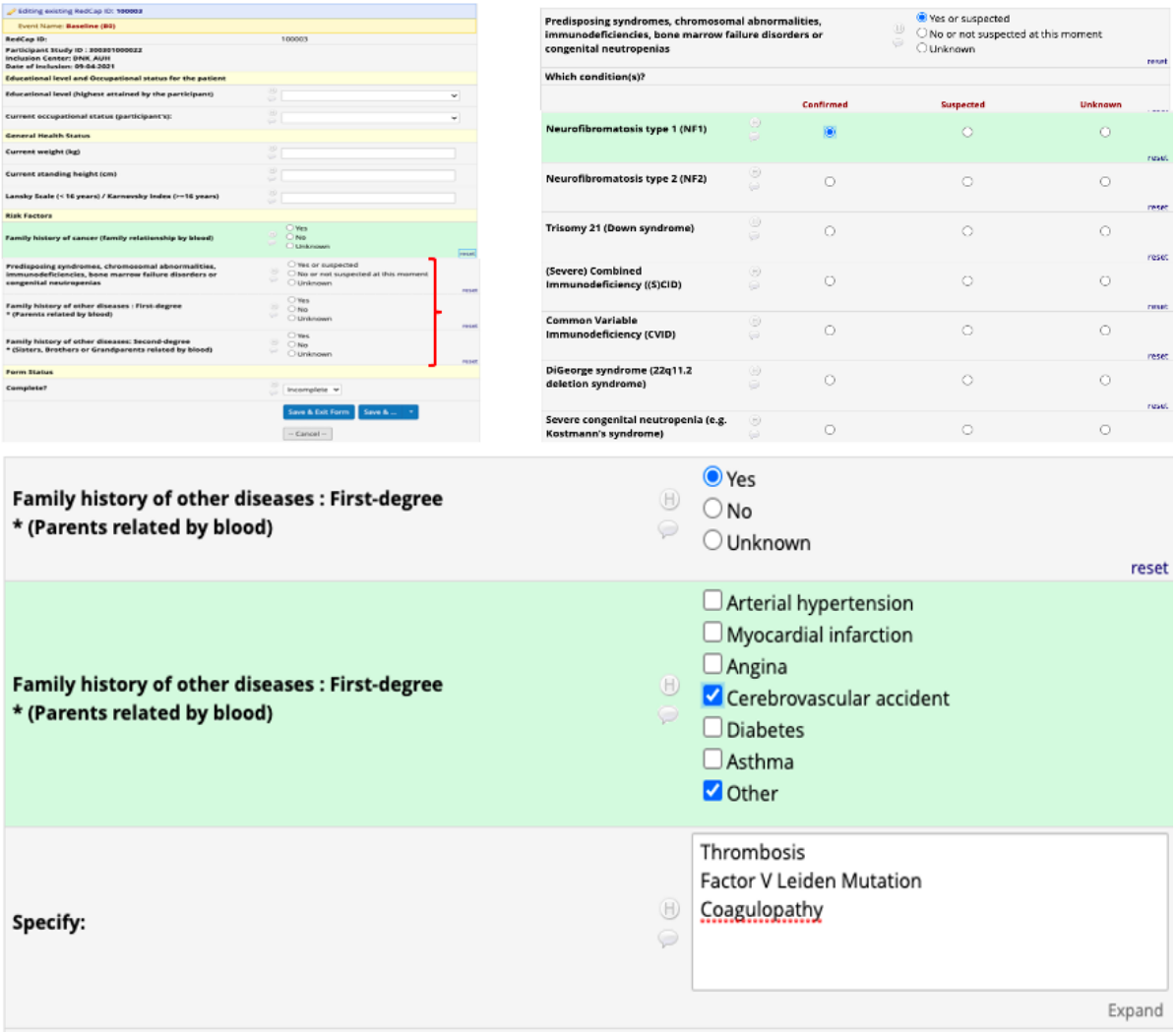

Figure VI. 5: Information on cancer predisposition syndromes and family history of other diseases. In this example, the patient is known with NF1 syndrome and the patient has a family history of other disease in the first-degree family, here for cerebrovascular accidents. Note that in the field other family history of deep vein thrombosis or of factor V Leiden or other familiar coagulopathies can be added if indicated. The field "family history of other diseases: Second-degree" should be filled out if siblings or grandparents have a family history.

3. Baseline_Known history of neurovascular disease in the patient

In the HARMONIC Redcap database baseline window, please find the BL&FU-Health/Adverse Events window, to indicate if the patient had in the past neurovascular diseases, this could be for example a stroke, an intracerebral haemorrhage, Moya Moya Syndrome etc. Also known comorbidities like hypertension, diabetes, atrial fibrillation etc are interesting for our task.

The Health Event can be dated or the age at which the participant experienced the event can be noted. If you do not know the date or the age, please still list the event.

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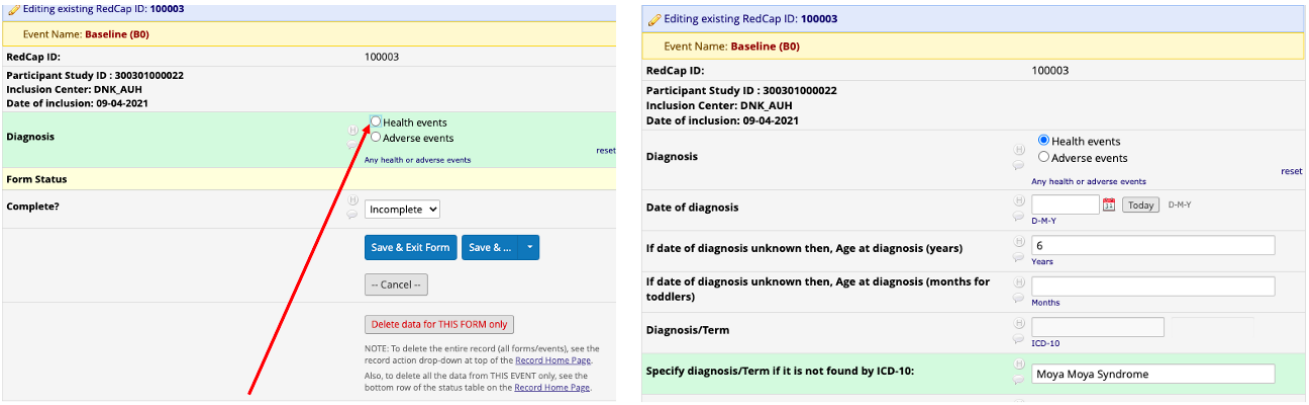

Figure VI.6: Information at baseline from the patient's medical history. In the example shown on the right side, the patient has developed Moya Moya Syndrome at the age of 6 years. If the diagnosis pops up in the ICD-10 under Diagnosis/Term you can fill the diagnosis out in the ICD-10 field.

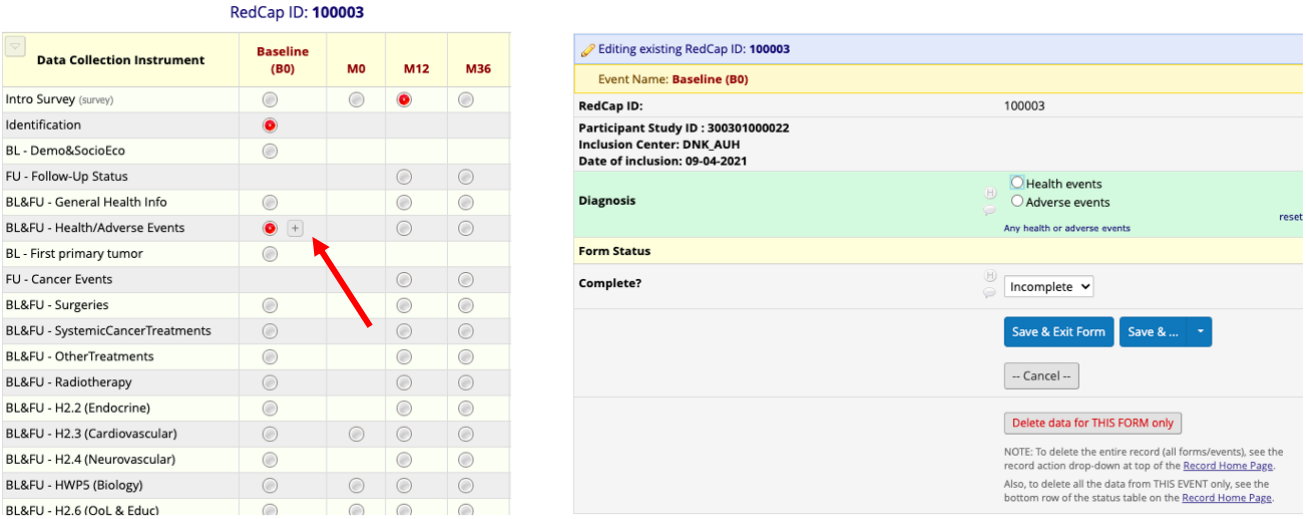

Figure VI.7: You can add several health events by clicking each time on the participant's dashboard + sign, this will open a new window with health and adverse events.

4. Baseline_Medication that can have influence on neurovascular morbidity

In the HARMONIC Redcap database baseline window, please find the BL&FU-OtherTreatments window. There you can indicate whether the patient is treated with anticoagulant medication, antiplatelet aggregation medication or hormonal contraception. If you tic yes, a window will appear where you can enter the duration of the treatment, if this is unknown, please still note the medication.

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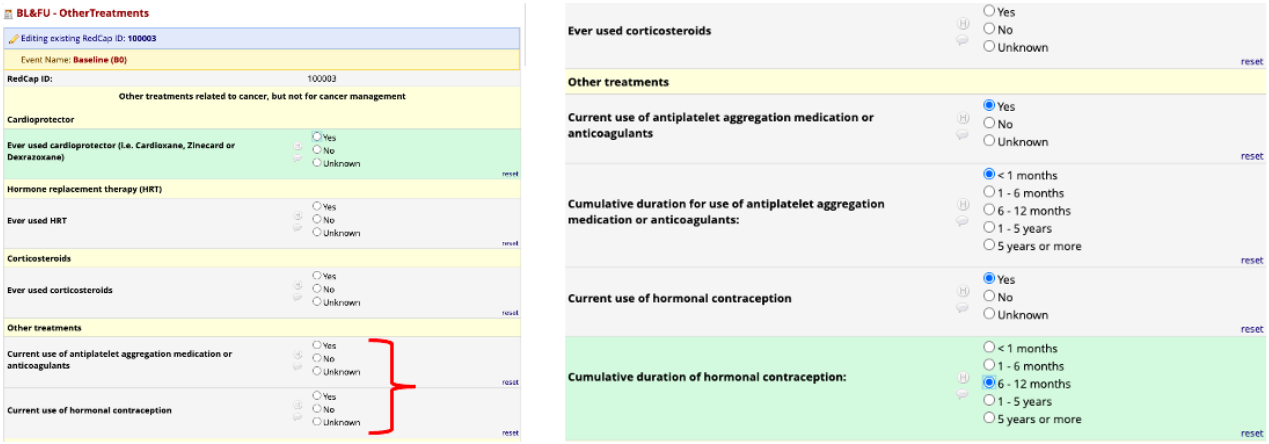

Figure VI.8: In the other treatment section, the current anticoagulant, antiplatelet aggregation and hormonal contraception are important for our task. In the example on the right side, the patient has taken Heparin for under a month and takes oral contraception for 9 months.

5. Baseline_Neurovascular symptoms and MRI image markers

In the HARMONIC Redcap database baseline window, please find the BL&FU-H2.4 (Neurovascular) window.

Here you will first find a list of neurological symptoms, often associated with precursors or manifest neurovascular disease. Multiple symptoms can be checked at the same time. Regarding headache an extra window will open, if none of these forms of headache apply, only check the headache box and leave the extra window blanc.

Then you will find a section for current diagnosis of neurovascular disease, which looks mainly after stroke, intracranial bleeding, TIA or PRIND. The diagnosis can either be made clinically or / and on imaging and there either on MRI or on CT. If you know the date of the diagnosis and of the imaging please add it, if not, leave it blanck.

The next section concern MRI image markers. Please indicate here the date of the pseudonymised Baseline MRI that has been uploaded to the Aquilab platform. Please upload the pseudonymised report of this MRI in this section.

The list with MRI image markers will be filled out by the radiologists from AUH, after review of the MRI on the Aquilab platform.

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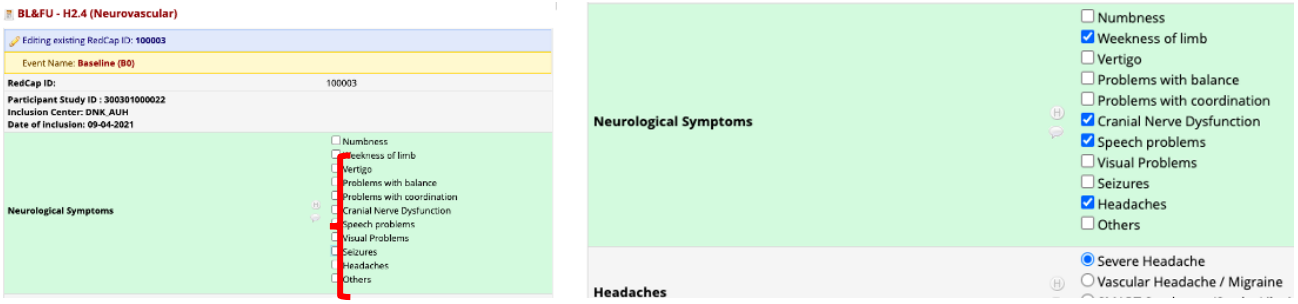

Figure VI.9: Symptoms often associated with neurovascular disease are listed, please check the boxes for what applies to the patient at baseline, leave it blanc if the patient does not have these symptoms. Multiple symptoms can be present in the same patient at the same time. When checking "headaches" a new drop down window will appear to define the kind of headache more precisely if indicated.

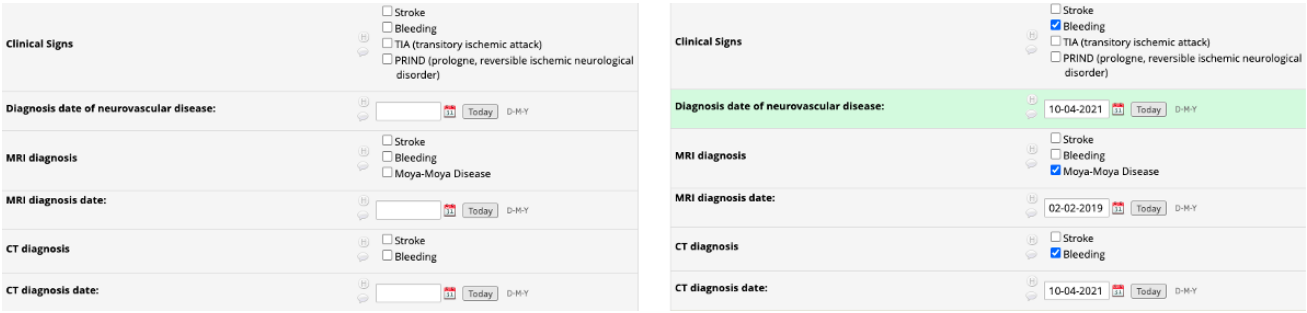

Figure VI.10: Fill this section out if the patient has a neurovascular disease at baseline either clinically or and seen on scans. See the right side as an example.

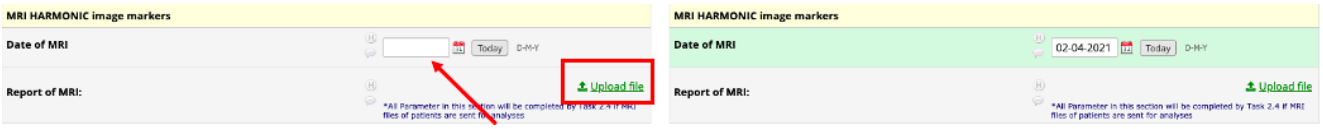

Figure VI.11: Note here the date of the Baseline MRI, upload the pseudonymized report of the MRI in the case below the date. Upload the pseudonymized MRI to the Aquilab platform by clicking the "upload file"box.

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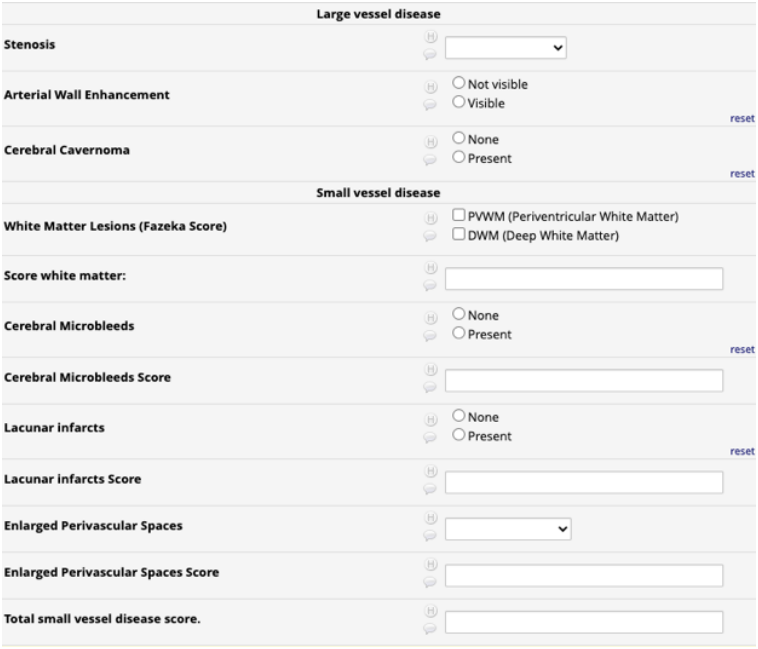

Figure VI.12: The list of image markers of the baseline MRI, this will be filled out by the AUH/AU team after review of the MRI on Aquilab.

6. Follow up, M12, M36, M60 or later

Select the correct Follow up window in the HARMONIC Redcap database. For every timepoint M12, M36, M60 or later the same parts of the eCRF need to be filled out for the neurovascular part.

In the M12 (M36/M60 etc) window, please find the BL&FU-General Health window, please fill out the risk factor section as described under point 2 of the annexe. Only fill this out, if something new here has occurred since the last timepoint in the database.

In the M12 (M36/M60 etc) window, please find the BL&FU-Health/Adverse Event window and please fill it out if neurovascular events have occurred since the last timepoint as described under point 3 of the annexe. If you think that the event is an adverse event fill it out as adverse event, if not fill it out as health event.

In the M12 (M36/M60 etc) window, please find the BL&FU-OtherTreatments window and update it regarding the patient's medication as described under point 4 of the annexe.

In the M12 (M36/M60 etc) window, please find the BL&FU-H2.4 (neurovascular) window and fill it out. Please list all the symptoms that the patient actually has at that timepoint. Only fill out the section of aggravation of symptoms if the patient already had a symptom before and it has now worsened. Fill out the section about neurovascular diseases found clinically or /and by imaging since the last timepoint as described under point 5 in the annexe. Note the date of the respective MRIs at M12, M36, M60 etc. and upload the respective pseudonymised report in the case below. Uplaod the pseudonymised MRI on the Aquilab platform. All other sections regarding MRI image markers and radiation doses will be filled out by the AUH/AU team.

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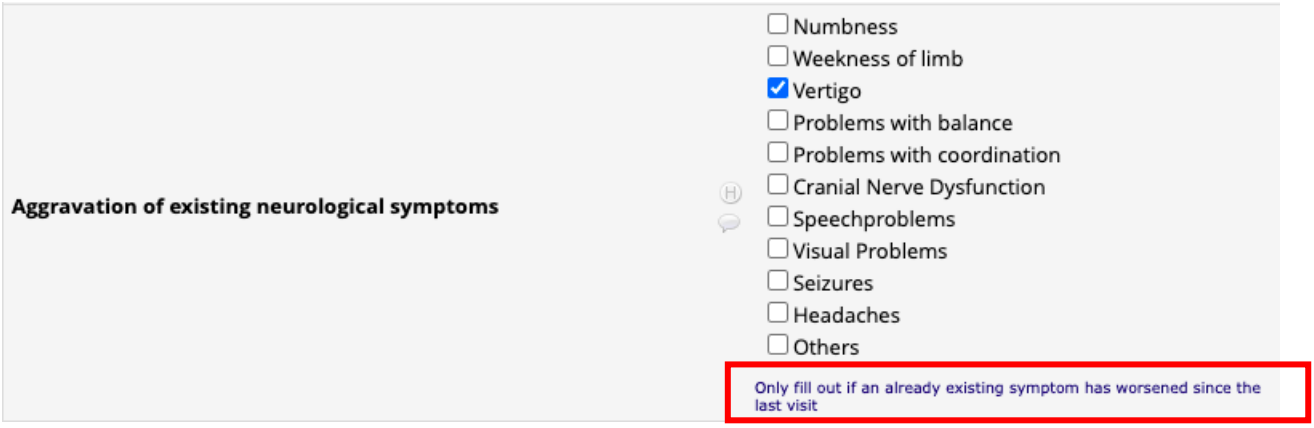

Figure VI.13: List of aggravation of existing neurological symptoms, only fill this out if an already existing symptom has worsened since last follow up. In this example the patient had vertigo at baseline and now at M12 it has worsened.

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Annex VI.2:

List of MRI markers and Radiation Dose-volume parameters extracted from the MRIs and radiation dose plans on the Aquilab platform and entered into the HARMONIC Redcap database by the AUH/AU team

1. Image markers

- Large vessel disease
  - Stenosis
    - No stenosis
    - < 50% stenosis
    - > 50% stenosis
    - Stenosis location
      - ICA, MCA, ACA, PCA, AComA, PComA, BA
  - Arterial Wall Enhancement
    - Not visible
    - Visible
    - If visible location
      - ICA, MCA, ACA, PCA, AComA, PComA, BA
- Cerebral Cavernoma
  - None
  - Present
    - Number of cavernoma
    - Location
      - Frontal, temporal, occipital/parietal, infratentorial, midbrain/sublobar, white matter, grey matter
- Small Vessel Disease
  - White Mater Lesions (Fazeka Score)
    - Perivenrtricular White matter
      - 0 (absent), 1 (caps of pencil thin lining), 3 (irregular periventricular signal)
    - Deep White Matter
      - 0 (absent), 1 (punctate foci), 2(beginning confluence), 3 (large confluent areas)
  - Cerebral Microbleeds
    - None
    - Present
      - Number of microbleeds
      - Location
        - ♦ Frontal, temporal, occipital/parietal, infratentorial, midbrain/sublobar, white matter, grey matter
  - Lacunar Infarcts
    - None
    - Present
      - Number of lacunar infarcts
      - Location
        - ♦ Frontal, temporal, occipital/parietal, infratentorial, midbrain/sublobar, white matter, grey matter
  - Enlarged Perivascular Spaces
    - None
    - < 10 (mild)

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- 10-25 (moderate)
- > 25 (severe)

➢ Total score Small Vessel Disease

2. Radiation Dose-Volume data

Dmean, Dmax, V%10,20,30,40,50Gy:

— Circle of Willis
— Suprasellar Cistern
— Chiasm
— Internal Carotid Artery ICA_L, ICA_R
— Middle Cerebral Artery 1st segment M1_L, M1_R
— Anterior Cerebral Artery ACA_L, ACA_R
— Posterior Cerebral Artery PCA_L, PCA_R
— Anterior Communicating Artery AComA_L, AComA_R
— Posterior Communicating Artery PComA_L, PComA_R
— Basilary Artery BA
— Brainstem

Dmean, Dmax, V%10,20,30,40,50Gy, Vcc 10,20, 30, 40, 50 Gy:

— Whole Brain
— Neurovascular pathologies

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Annex VI.3:

Contacts in the different centres relevant for the neurovascular task

Institution	Contacts
AUH/AU (Denmark)	Yasmin Lassen (yasmin.lassen@auh.rm.dk) Morten Høyer Sanja Karabegovic Ronni Mikkelsen Louise Tram Henriksen Laura Toussaint Dorte Winther Annette Krabbe Gade Jane Hagelskjær Knudsen
Centre François Baclesse (France)	Charlotte Demoor Camille Vidaud Juliette Thariat Agnes Dumas
Gustave Roussy (France)	Brice Fresneau Stephanie Bolle Nadia Haddy Nathalie Boddaert (Hôpital Necker) Volodia Dangouloff-Ross (Hôpital Necker)
KU Leuven (Belgium)	Sofie Isebaert Karin Haustermans Charlotte Sleurs Sandra Jacobs Karen Van Beek Ben Verhaaren Ronald Peeters Inge Witt
UK Essen (Germany)	Beate Timmermann Sophia Göricke Martina Wette
Task 2.6	Agnes Dumas
Task WP5	Siamak Haghdoost Nadia Haddy
Inserm	Neige Journy Thi-Chien Tran
ISGlobal	Isabelle Thierry-Chef

Table VI. 2: Contacts in the different centres associated with the neurovascular task

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VII. Task 2.5 Second Cancers after photon and proton beam therapy

1. The purpose of this Standard Operating Procedure (SOP) is to provide more specific information and guidance on which data need to be collected related to WP2 Task 2.5 Second Cancers: to indicate how to collect data and how to enter data on second and any subsequent primary cancers (SPC) into the HARMONIC clinical database developed through REDCap software. This way, harmonized data collection across all recruiting clinical centers will be guaranteed in order to ensure high quality data collection and analysis.

   - SOP I – Participant Study ID
   - SOP III WP5
   - SOP IX -Dosimetry/Data transfer Aquilab
   - HARMONIC eCRF
   - Guidelines for data entry in the clinical database

COUNTRY	INVESTIGATING CENTRE	ROLE IN TASK 2.4	PRINCIPAL INVESTIGATOR
Belgium	Department of Radiation Oncology, UZ Leuven	Leader	Prof Dr. Karin Haustermans
France	INSERM	Leader	Dr Neige Journy
	CRFB - Centre François Baclesse, Service Radiothérapie	Partner	Prof Dr. Juliette Thariat
	Gustave Roussy (GR) Department of Radiation Oncology	Partner	Dr. Stéphanie Bolle
Denmark	AU - Danish Centre for Particle Therapy | AUH - Aarhus Universityhospital	Partner	Dr. Yasmin Lassen
Germany	Universitätsklinikum Essen (AöR) Klinik für Partikeltherapie Westdeutsches Protonentherapiezentrum Essen (WPE) Partnerstandort im Deutschen Konsortium für Translationale Krebsforschung	Partner	Prof Dr. Beate Timmermann
Netherlands	Princess Maxima Center (PMC)	Partner	Dr Judit Kok
	University Nedical Cneter Gorningen (UMCG)	Partner	Dr John Maduro
Norway	Oslo University Hospital (OUS)	Partner	Dr Kristina Kjaerheim
Switzerland	University of Zurich (UZH)	Partner	Dr Linda Walsh

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- All children, adolescents and young adults treated with photon or proton beam therapy at age ≤ 18 years[1] for any cancer type;
- For both the retrospective and prospective cohorts.

5.1 Requirements

- For data collection: prospective or retrospective patient data; where possible, linkage with external registries (e.g. national/regional cancer registries) or databases (e.g. hospital claims, healthcare reimbursement systems)

- For data entry:
  - REDCap database
  - ICD-O-3

5.2 Definition and format of SPC data collected in HARMONIC-RT

Table VII.4. Overview of SPC parameter definitions and formats

#	Parameter	Definition/format	Level
1	Date of diagnosis	Date on which the SPC was diagnosed: dd/mm/yyyy	1
2	Age at diagnosis	Age of the patient at the moment of the SPC diagnosis; Calculated automatically based on date of diagnosis – only to be filled in (in years or months) if date of diagnosis is unknown	1
3	Localization	According to ICD-0-3 (topography)	1
4	Laterality (for paired organs only)	Left, right, one side (if left/right unknown), bilateral, unknown, not applicable	2
5	Histology	According to ICD-0-3 (morphology-behaviour)	1
6	Grade	Well differentiated Moderately differentiated Poorly differentiated Undifferentiated or anaplastic T-cell – T-precursor cell B-cell – B-precursor cell Null cell – Non-T-non-B Natural Killer (NK) cell I (WHO grading system) II (WHO grading system) III (WHO grading system) IV (WHO grading system) If other, specify Unknown – not stated or not applicable	1

[1] under 22 years for France only

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#	Parameter	Definition/format	Level
7	Staging system	Toronto guidelines - Tier 1 Ann Arbor Cotswolds revision of the Ann Arbor St Jude/Murphy staging system IRS (Inter-group Rhabdomyosarcoma)-modified TNM stage COG staging system for acute lymphoblastic leukaemia Abbott et al. Leukemia 2003, for acute myeloid leukemia COG/NWTSG (National Wilms Tumour Study Group) staging system (prechemotherapy only) SIOP staging system M-staging system Chang's staging system for medulloblastoma FIGO (International Federation of Gynaecological Oncologists) staging system for ovarian cancer INRGSS (International Neuroblastoma Risk Group Staging System) INSS (International Neuroblastoma Staging System) IRSS (International Retinoblastoma Staging System) IRS (Inter-group Rhabdomyosarcoma) grouping system Toronto guidelines for soft tissue and bone sarcoma (based on the TNM classification) pTNM staging system (pathological stage) TNM staging system (clinical stage) Toronto guidelines for testicular cancer (based on the TNM classification) PRE-Treatment EXTent of tumor (PRETEX) Other staging systems, please specify (Free text)	1
8	Stage at diagnosis	Depends on the Staging system	1
9	Diagnostic procedures	Histology Cytology Radiology Specific tumour markers Clinical investigation or medical record Autopsy Certificate of death Health insurance database Disease registry, please specify Unknown	1
10	Molecular subgroup (when relevant)	Medulloblastoma group I (WNT) Medulloblastoma group II (SHH) Medulloblastoma group III Medulloblastoma group IV TP53 mutation MYC/N-MYC amplification c-MYC amplification Elevated AFP serum or CSF Elevated β-HCG serum or CSF Segmental chromosomal alteration Numeric chromosomal alteration ALK aberration PAX3 or PAX7 – FOXO1 fusion MYOD1 mutation PFA (H3K27M methylation) PFB, ST – RELA fusion ST – YAP1 fusion SC – MYCN amplification	2

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#	Parameter	Definition/format	Level
		BRAF v600E mutation H3K27M mutation or loss of H3K27M methylation H3 G34 mutation IDH1/IDH2 mutation 1p19q codeletion ATRX mutation Other: please specify Unknown - not stated or not applicable	
11	**Localization of SPC with respect to the treated volume**	Within the treated volume (i.e. volume encompassed by the D98% isodose) In margin of the treated volume Out of the treated volume (including leukemias) Unknown	2

Level 1: mandatory item; level 2: optional item

#1 Date of diagnosis

The date of diagnosis can be defined, according to descending priority, as:

1. Date of first microscopic confirmation of malignancy (histology, cytology…)
    a. Date of collection of biopsy/cytology
    b. Date of receipt of biopsy/cytology by the pathologist
    c. Date of pathology report
2. Date of first hospitalization for cancer
3. Date of first consultation for reasons of malignancy (if no hospitalization/ambulatory)
4. Date of clinical or technical investigation
5. Date of start of treatment for cancer
6. Date of death (if no other information available)

#2 Age at diagnosis

To be registered only if the date of diagnosis is unknown; otherwise, this will be automatically provided by REDCap.

#3 Localization

Use ICD-O-3.2 whenever it is possible; otherwise, use ICD-O-3.1 or ICD-10 if needed

Resources:
http://www.iacr.com.fr/index.php?option=com_content&view=category&layout=blog&id=100&Itemid=577

Remark: as long as ICD-O-3 is not available in REDCap, ICD-10 is used as ICD-O-3T is the same as ICD-10 for topography

#4 Laterality

It should be registered for paired organs only, e.g eye, lung, breast, gonads, limbs.

#5 Histology

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Use ICD-O-3.2 to register morphology – behaviour whenever it is possible; otherwise, use ICD-O-3.1

Resources:
http://www.iacr.com.fr/index.php?option=com_content&view=category&layout=blog&id=100&Itemid=577

Remark: as long as ICD-O-3 is not available in REDCap, a free text field will be available to enter the morphology and behaviour codes as follows: mmmm-b

#6 Grade

Select the appropriate grade according to the pathology.

#7 Staging system (for registration of cancer stage at diagnosis)

The definition of staging system for diagnostic group of cancer follows the Toronto Paediatric Cancer Stage Guidelines which is endorsed by the IARC and other Guidelines (Please see Annex VII. 3).

#8 Stage at diagnosis

The definition of cancer stage at diagnosis follows the Toronto Paediatric Cancer Stage Guidelines which is endorsed by the IARC (Please see Annex VII.3).

#9 Diagnostic procedure

Indicate the diagnostic procedure(s) used to diagnose the SPC.

#10 Molecular subgroup

This item will be regularly upgraded in the next versions of the database, depending on the data provided by the investigators in the pilot phase of the registry and latest advances in the understanding of oncogenesis and in clinical practice.

#11: Localization of SPC with respect to the treated volume

The treated volume (TV) is defined as the volume encompassed by the D98% isodose.* A SPC within the TV is a true "in-field" SPC due to inadequate dose (or inadequate time-dose pattern) or treatment delivery. A SPC adjacent to the TV is considered a "marginal" SPC due to inadequate volume delineation (wrong evaluation of the CTV and/or PTV) or a mistake in treatment delivery.

* ICRU report 50 (1993), report 59 (2007) and report 83 (2010)

Because of the limitations of irradiation techniques, the volume receiving the prescribed absorbed dose (i.e. treated volume, TV) might be different than the planning target volume (PTV); it might be larger (sometimes much larger) or smaller, and in general more simply

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shaped (less so with IMRT than with conventional or three-dimensional radiation therapy). The TV is the volume of tissue enclosed within a specific isodose envelope, with the absorbed dose specified by the radiation oncology team as appropriate to achieve tumor eradication or palliation, within the bounds of acceptable complications. As proposed in proton therapy (ICRU, 2007), D98 % could be selected to determine the TV in photon therapy.

5.3 Methods and timeline for data collection

SPC data is collected both through active or passive follow-up of the study participants.

Active follow-up

The investigator should record any diagnosis of SPC which he/she is aware of, whether it was diagnosed at his/her center or not. The information asked on SPC in the HARMONIC project is similar as to what is being asked for in (inter)national paediatric cancer registries. SPC information should be recorded at the time of a study follow-up timepoint (M12, M36, etc), or at any time according to routine visits for patient care. In case SPC information is recorded independently of the study follow-up timepoints, it should be recorded as a new "FU - Cancer Events" form which is added to the latest study timepoint.

Passive follow-up

Whenever it is possible, the HARMONIC principal investigators should seek for agreements with coordinators of external registries (e.g. national/regional cancer registries) or databases (e.g. hospital claims, healthcare reimbursement systems) to link them with the HARMONIC database related to their patients. Such linkage should enable the investigators to collect detailed information on SPC diagnoses (+possibly, on SPC treatments) through a passive follow-up of the study participants. Passive follow-up adds very important information to the active follow-up regarding to the SPC analyses because it would provide a very long-time follow-up of the study participants after cancer treatment, irrespective of their health status and socioeconomic considerations. Therefore, it would allow providing unbiased estimates of the SPC incidence.

Linkage should be done for all included patients (including those who withdrew from the study, died or were lost of follow-up by the investigating center), preferably **at least once by the end of 2023** (afterwards, every two years if possible; the frequency of linkage may vary across the centres depending on local considerations). Linkage will be done for the local or national cohorts **by the investigating center or an authorized entity** at the national level (i.e. Inserm for French patients only), respectively.

Data access from external registries or 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 2). The national civil identification / health insurance number are obtained in medical records when retrospective inclusion or directly from the participant at the

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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 HARMONIC database by the investigating center or authorized entity.

Methods for linkage: To be defined in each country, depending on the procedures agreed on with the coordinators/administrators of the external registries or databases. The potential costs occurring for linkage should be covered by the investigating centres or authorized entity at the national level.

Table VII.5. 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
Disease registries (long-term endpoints)			
Belgium	Belgian Cancer Registry	INSZ	Exact matching
Denmark	Danish Childhood Cancer Registry	tbd	tbd
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)			
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			

Any questions related to this SOP and the procedures described herein can be addressed to: Neige.JOURNY@gustaveroussy.fr, karin.haustermans@uzleuven.be, sofie.isebaert@uzleuven.be, inge.2.dewit@uzleuven.be, or karen.vanbeek@uzleuven.be

For questions about REDCap, contact Thichien.TRAN@gustaveroussy

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Annex VII.1: Additional information for researchers involved in Task 2.5 Second Cancers

BACKGROUND

Second and subsequent primary cancer (SPC) 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 (Central Nervous System (CNS), thyroid, breast, lung, gastrointestinal organs and tracts, soft tissues and bones), leukemia and myelodysplasia (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 [1-3].

Young patients are specifically prone to developing 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 relative 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 [2,3];

- For a given radiation dose, childhood and adolescents are more susceptible to develop radiation-related cancers than adults [1-4]. There is strong evidence of an increased risk per Gy with decreasing age at exposure, for leukaemia (other than chronic lymphocytic leukaemia), breast, brain and thyroid cancers, and moderate evidence for other cancer sites such as non-melanoma skin, bladder and stomach cancers [4]. This higher susceptibility to ionizing radiation relates to a number of developmental and physiological differences between children, adolescents and adults. In addition, a larger proportion of paediatric tumours (8.5% in a large case series [5]) compared to adult tumours 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 [6].

Subsequently, SPC 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 [7]. Children and adolescents treated with cranial radiotherapy had a 10 times higher risk to develop a subsequent CNS tumour compared to their siblings [8]. Among females who were treated with chest radiotherapy before the age of 21 years, the cumulative incidence of breast cancer was 30% [9] - 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 [1], 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 [10], normal tissue irradiated

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volumes and dose gradients [11], and beam qualities 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 external beam radiotherapy (EBRT) techniques on second primary cancer risks has not been quantified in clinical studies.

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 [12]. 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-mentioned factors, measured biomarkers of carcinogenesis, and a long-term, standardized follow-up of patients with both active and 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.

AIMS

General aim

To develop a concept and methods for analyses on second cancer risk after EBRT with different techniques, including proton and photon beam therapy.

Specific aims

- We will review referral guidelines and practices for proton and photon beam therapy to get more insight into the presumed indication bias in the referral pattern for proton therapy.

- We will compare clinical and socioeconomic characteristics of paediatric patients treated with different EBRT techniques in the HARMONIC WP2 database, but also in other external databases which are representatives of the general population (e.g. SNIIRAM and PMSI for France[2]). These preliminary analyses will enable us characterizing and quantifying the presumed indication bias in comparative studies on

[2]Access pending authorizations by the competent regulatory authorities.

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proton vs photon beam therapy in children and adolescents, and defining appropriate statistical methods for dose-volume-risk analyses accounting for indication bias (i.e. subgroup, adjusted, and/or individually matched-pair analyses).

— Last, we will propose statistical methods for matched dose-volume risk analyses accounting for indication bias in anticipated future studies on SPC risks (and in cost-effectiveness 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 there is a sufficient patient follow-up time and register sufficient number of cases to study outcomes that usually occur >10 years after treatment.

INCLUSION CRITERIA

— All children, adolescents and young adults treated with photon or proton beam therapy at age ≤ 18 years[3] for any cancer type

— from both the retrospective and prospective cohorts.

STUDIED PARAMETERS

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 accounting for sex, attained age, time since exposure and other confounding factors (cf. Annex 2 for more details).

Cancer sites to be investigated

The following cancer sites will be primarily investigated: CNS, thyroid, breast, lung, gastrointestinal organs and tracts and genitourinary organs and tracts. However, investigations for other cancer sites are not excluded.

Soft tissues, bones, skin and active marrow are important tissues to consider regarding SPC risk, but challenging in terms of dosimetry. We will also consider the stage at diagnosis and the procedures/methods of SPC diagnosis to detect potential surveillance biases.

Relevant organs at risk

Table VII.3. Relevant OAR for SPC

SPC sites to be investigated	OAR
CNS	Whole brain

[3] under 22 years for France only

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	Spinal Cord
Thyroid	Thyroid gland
Breast	Breasts
Lung	Lungs
Gastrointestinal organs and tracts	mainly esophagus, stomach wall, liver, bladder, colon, small intestine)
Genitourinary organs and tracts	Mainly testes, ovaries, and kidneys

More information on contouring and dose reconstruction will be provided in WP4 Dosimetry. The idea is to contour as much as possible the organs of interest in CT images (the option of automatic contouring is being explored) for organs located within or at the margin of the RT field. If they cannot be contoured, the estimation made by the analytical model on the phantoms will be used instead.

Radiation related factors

- Radiation-related factors: e.g. total dose, dose fractionation, irradiated volume of the organ, beam quality,…

Risk factors to be investigated

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), age at irradiation
- Lifestyle characteristics: smoking history, alcohol consumption, drug consumption, physical activity/sedentariness
- Hormonal factors: sex-specific factors (e.g. age at puberty), comorbidities, treatments

Other relevant information to be investigated

- Patient identification & follow-up information
- Primary tumour information & follow-up information
- Other health events in follow-up
- Referral guidelines and practices for proton vs. photon beam therapy will also be reviewed to get more insight into the presumed indication bias.

Statistical analysis plan

The analyses will be performed 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.

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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.

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Annex VII.2: Overview relevant variables task 2.5

SPC specific info		
Category	Subcategory	Remark
Category	Subcategory	Remark
SPC information	Date of diagnosis	
SPC information	Age at diagnosis	
SPC information	Localization	ICD-10
SPC information	Laterality	
SPC information	Histology	ICD-0-3 (morphology)
SPC information	Grade	
SPC information	Staging system	
SPC information	Stage at diagnosis	
SPC information	Diagnostic procedures	
SPC information	Molecular subgroup (if relevant)	
SPC information	Extent of SPC with respect to treated volume	

Clinical factors	Family history of cancer	
Category	**Subcategory**	**Remark**
	Predisposition syndromes, chromosomal abnormalities, immunodeficiencies, bone marrow failure disorders, congenital neutropenias	
	Comorbidities (healt/adverse events)	ICD-10
	Non-cancer medications	
	Age	
	Sex	
	Obesitas (weight/heigth)	
Local/Systemic Cancer Treatments	Chemotherapy	
	Immunotherapy	
	Hormonal therapy	
	Targeted therapy	
	Surgery	
Lifestyle characteristics	Smoking status	
	Alcohol consumption	
	Drug consumption	
	Physical activity/sedentariness	Questionnaires about fatigue
	Environmental factors	Place of residence
Hormonal factors (Sex-specific)	Age at puberty	
	Comorbidities	
	Treatments	

Radiation factors		
Category	**Subcategory**	**Remark**
Age at treatment		Time since exposure
Treated organ/area		
Protocol		
Target(s)		
Beam quality		
Delivery technique		
No. Beams		
Adaptive replanning		Additional imaging exposure
Total dose		
Dose fractionation		
Conditions of irradiation		Imaging for positioning verification
Replanning during treatment		Additional imaging exposure

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Other relevant information		
Category	**Subcategory**	**Remark**
Patient Identification & Follow-up	Study Participant ID (Registry level)	
	Inclusion: Center, country	
	Inclusion: Status (i.e. prospective, retrospective)	
	Inclusion: Date of patient registration (dd/mm/yyyy)	
	Last in-/out-patient visit at the inclusion centre: Date (mm/yyyy)	
	Date of last contact/follow-up information with resepct to vital status	
	Vital status	
	Date of death	
	Main cause(s) of death	
First primary cancer	Date of diagnosis	
	Age at diagnosis	
	Diagnostic procedures	
	Localization	ICD-10
	Laterality	
	Histology	ICD-0-3 (morphology)
	Grade	
	Staging system	
	Stage at diagnosis	
	Molecular subgroup (if relevant)	
FUP first primary cancer	Recurrence/progression	
	Date of recurrence/progression	
	Extent of recurrence/progression	
Health/Adverse events (Follow-up)	Other disease	

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Annex VII.3 Staging system / Stage at diagnosis

Staging system	Stage at diagnosis	References
Toronto guidelines - Tier 1	Localized, Locoregional, Regional, Metastatic, MS disease, Limited, Advanced, Unknown	http://www.iacr.com.fr/index.php?option=com_content&view=article&id=153&Itemid=657
Ann Arbor	I, IA, IB, II, IIA, IIB, III, IIIA, IIIB, IV, IVA, IVB, Unknown	
Cotswolds revision of the Ann Arbor	I, IE, II IIE, IIIS(1), IIIE(2), IIISE, IV, Unknown	https://radiopaedia.org/articles/cotswolds-modified-ann-arbor-classification-2
St Jude/Murphy staging system	I, II, III, IV, Unknown	http://www.iacr.com.fr/index.php?option=com_content&view=article&id=153&Itemid=657
IRS (Inter-group Rhabdomyosarcoma)-modified TNM stage	1, 2, 3, 4, Unknown	https://www.cancer.net/cancer-types/rhabdomyosarcoma-childhood/stages-and-groups Brierley J, Gospodarowicz M, Wittekind C, eds. The TNM Classification of Malignant Tumours, 8th edition. Hoboken, NJ: John Wiley and Sons Inc, 2017
COG staging system for acute lymphoblastic leukaemia	CNS-, CNS+, CNS1, CNS2, CNS3, Unknown	http://www.iacr.com.fr/index.php?option=com_content&view=article&id=153&Itemid=657
Abbott et al. Leukemia 2003, for acute myeloid leukemia	CNS-, CNS+, Unknown	
COG/NWTSG (National Wilms Tumour Study Group) staging system (prechemotherapy only)	I, II, III, IV, Unknown	
SIOP staging system	I, II, III, IV, Unknown	
M-staging system	M0, M1, M2, M3, M4, Unknown	
Chang's staging system for medulloblastoma	T1, T2, T3a, T3b, T4, M0, M1, M2, M3, M4, Unknown	http://www.bioline.org.br/pdf?ni06003
FIGO (International Federation of Gynaecological Oncologists) staging system for ovarian cancer	I, II, III, IV, Unknown	http://www.iacr.com.fr/index.php?option=com_content&view=article&id=153&Itemid=657
INRGSS (International Neuroblastoma Risk Group Staging System)	L1, L2, M, MS, Unknown	
INSS (International Neuroblastoma Staging System)	1, 2A, 2B, 3, 4, 4S, Unknown	
IRSS (International Retinoblastoma Staging System)	0, I, II, III, IV, Unknown	
IRS (Inter-group Rhabdomyosarcoma) grouping system	I, II, III, IV, Unknown	https://www.cancer.net/cancer-types/rhabdomyosarcoma-childhood/stages-and-groups
Toronto guidelines for soft tissue and bone sarcoma (based on the TNM classification)	I, II, III, IV, Unknown	http://www.iacr.com.fr/index.php?option=com_content&view=article&id=153&Itemid=657

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pTNM staging system (pathological stage)	T0, T1, T2, T3, T4, Tx, N0, N1, N2, N3, Nx, M0, M1, Mx, Unknown	
TNM staging system (clinical stage)	T0, T1, T2, T3, T4, Tx, N0, N1, N2, N3, Nx, M0, M1, Mx, Unknown	
Toronto guidelines for testicular cancer (based on the TNM classification)	I, II, III, Unknown	
PRE-Treatment EXTent of tumor (PRETEX)	I, II, III, IV, Unknown	https://www.pedrad.org/Portals/5/Subspecialties/Abdominal%20Imaging/PRETEXT%202017.pdf?ver=2018-07-09-155954-130
Other staging systems, please specify (Free text)	Other stages at diagnosis , please specify (Free text)	

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VIII. Task 2.6 - Quality of life & academic achievement (QoL)

This Standard Operating Procedure (SOP) has been written to provide specific guidance and precise information on the procedures to collect the data related to quality of life and academic achievement in HARMONIC study.

The procedures described in this document will harmonize the collection across all the recruiting clinical centres, with the purpose to obtain data of high quality, decreasing the potential biases and clinical centre's effects that could impact negatively on the interpretation of results. That is why it is of utmost importance that all persons involved in QOL data collection read it carefully to understand every procedure in a very clear manner. Updates to this document should be maintained throughout the life of the study to reflect any changes in data management procedures.

- HARMONIC protocol v1.0
- HARMONIC CRF
- SOP I - Participant Study ID

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4.1 Rationale and objective of the study

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 (QoL). The aim of the HARMONIC project is thus to investigate medium- and long term QoL, as well as social and academic outcomes in paediatric patients treated with modern EBRT techniques, and identify clinical and socioeconomic determinants related to them.

Design of the study: this is a prospective multicentre study

4.2 List of recruiting centres

Investigating Country		Investigating centre		Country Centre Code (HARMONIC REDCap)	Country Centre Code (HARMONIC REDCap)
Name	Code	Name	Code		
BELGIUM	100	Department of Radiation Oncology UZ Leuven Belgium	101	BEL_KUL	100101
DENMARK	200	Danish Centre for Particle Therapy | Aarhus Universityhospital Denmark	201	DNK_AUH	200201
GERMANY	300	Universitätsklinikum Essen (AöR) Klinik für Partikeltherapie Westdeutsches Protonentherapiezentrum Essen (WPE) Partnerstandort im Deutschen Konsortium für Translationale Krebsforschung Germany	301	DEU_ESSEN	300301
FRANCE	400	Centre François Baclesse Service Radiothérapie France	401	FRA_CRFB	400401

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5.1 Inclusion criteria

- Patient being 2 years old and aged less than 18 years old at first EBRT
- Patients and/or parent(s)/guardian(s) willing and able to comply with fulfilling questionnaires (at the investigator's discretion)
- Signed informed consent

5.2 Non-inclusion criteria:

Patients included in the following trials are excluded from the HARMONIC study:

- CoALL-97/AML-BFM 98 follow-up
- AML-BFM 2004 / 2012
- ALCL 99 international protocol
- Euro-LB 02
- Weichteilsarkome
- EURAMOS-1
- EWING 2008
- Nephroblastome: Nachsorge und Diagnostik von Spätfolgen
- MAKEI 96 / MAHO 98-follow-up
- MAKEI 2005
- NPC-2003-GPOH
- SIOP CNS GCT II
- HIT-2000
- HIT-HGG 2007
- Kraniopharyngeom 2000

5.3 Withdrawal

- Death of the patient
- The participant reached the age of 25
- For France, Denmark and Belgium: Minor participant reaching the age of majority, who did not sign a new informed consent (this will be interpreted as a decision to withdraw from the research)
- Termination of the participant's participation at the investigator's discretion (for any reason)
- Patient/ parent(s)/guardian(s) withdrawal from the research (for any reason)

5.4 New consent to be signed when the patient reaches age 18

In France, Denmark and Belgium, the ethical protocol requires that all patients becoming major (e.g. aged 18 or older) sign a new consent form to participate to the study **and gives his/her own personal address mail to receive the QoL questionnaires by email**. If the patient does not have a personal email address, the email of the parent can still be used.

The questionnaires consist of QoL assessment (generic core scale – 23 items) and multidimensional fatigue (18 items), through the PedsQL™, which is designed depending on the age of the child/adolescent; and questions about academic achievement and potential key confounding factors.

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The questionnaire is either proposed to the patient ("self-report") and/ or the parent ("proxy-report"), depending on the age of the patient. 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.

Attained age of the patient	Individuals invited to fill in the questionnaire	Name of the paper questionnaire
2 to 4 (Toddler)	Parent	Harmonic_par_incl_2to4
5 to 7 (Young Child)	Parent + self-report	Harmonic_par_incl_5to7 Harmonic_child_incl_5to7
8 to 12 (Child)	Parent + self-report	Harmonic_par_incl_8to12 Harmonic_child_incl_8to12
13 to 17 (Adolescent)	Parent + self-report	Harmonic_par_incl_13to17 Harmonic_ado_incl_13to17
18 to 25 (Young Adult)	Self-report	Harmonic_ya_incl_18to25

Questionnaires are completed over a maximum of a 10-year period after cancer diagnosis (and not after age 25), with 6 timepoints:

- Inclusion: before EBRT
- M0: immediately after or within 3-6 months after completion of radiotherapy
- M12: 1 year after completion of radiotherapy
- M36: 3 years after completion of radiotherapy
- 5 and 10 after cancer diagnosis

The questionnaires are either completed during clinic visits (paper-based questionnaires), or at home (electronic online questionnaires). Online questionnaires are automatically sent to the patients once the starting date of data collection (M0, end of treatment) has been entered into REDCap.

Time point	Type of questionnaire
Inclusion (before treatment)	Paper questionnaire
M0: last day of radiotherapy (3-6 months following the last fraction of radiotherapy)	Paper questionnaire for patients with biological data collection OR who are seen at the centre Online questionnaire for other patients
M12 (1 year after the end of treatment)	Online questionnaire
M36 (3 years after the end of treatment)	Online questionnaire
+ 5 years after date of inclusion (baseline)	Online questionnaire
+ 10 years after date of inclusion (baseline)	Online questionnaire

8.1 Instructions for paper-based questionnaires

The study coordinator must fill the **first page of the questionnaire** with the date, the Study subject code (defined by the centre) and **Participant study ID** (defined by REDCap).

The guidelines for completing the QoL questionnaires are described below. An effort should be made to follow them, but this might not be feasible in every center:

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If feasible, the QoL questionnaire should be completed before the respondents complete any other health data forms and before they see their physician or healthcare provider.

If feasible, the parent and child must complete the questionnaires **independently of one another**. Discourage the parent, child, or other family members from consulting with one another during the completion of the questionnaire. If you are administering the questionnaire to the child, the child should be facing away from the parent.

If feasible, **children aged 7 and lower should be administered the questionnaire by the study coordinator**, reading the instructions and each item to the young child word for word.

- At the beginning of each subscale repeat the recall interval instructions to remind the young child to respond only for that specific recall interval. Use the separate page with the three faces response choices to help the young child understand how to answer. When reading items aloud to a child, intonation should be kept neutral to avoid suggesting an answer.
- If a child has difficulty understanding the age-appropriate questionnaire, the preceding age group version may be administered to the child (e.g., administering the Young Child (5-7) Self-Report version with the three faces response choices to an 8-year-old).

If a child presents **severe impairments** (as determined by the administrator), only the Parent-Proxy-Report should be administered to the child's parent.

If the child or parent has a question about what an item means or how they should answer it, do not interpret the question for them. Repeat the item to them verbatim. Ask them to answer the item according to what they think the question means. If they have trouble deciding on an answer, ask them to choose the response that comes closest to how they feel. The child and/or the parent has the option of not answering a question if they truly do not understand the question.

8.2 Once the paper questionnaire is completed

1. When the parent/child returns the questionnaire, look it over and check to see that all answers have been completed. If any responses are incomplete, illegible, or there are multiple responses for an item, please ask the parent or child to indicate their response if you have the opportunity to do it.
2. Ask the participants if they had any difficulties completing the questionnaire or if they have any other comments regarding the questionnaire. Document any important feedback.
3. Thank the parent and child for taking the time to complete the questionnaire. **Let them know that they will be asked to complete the questionnaire regularly (approximately every 2 years) and that they will be sent a link to their email address to complete it online.**
4. Enter manually the data reported by the patients and/or his parent on the paper-based questionnaires **into REDCAP.**

8.3 Instructions for online questionnaires

Study investigators have to enter manually the e-mail address of participants into the REDCAP system (local database), as well as a set of data (please see the section below "Screenshots of REDCAP") to enable (or stop) the sending of online questionnaires.

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An automatic invitation to complete the questionnaires online on the REDCAP secured platform is sent at home by regular email to the parents (or patients) who provided their email addresses to the investigator, except in the case of withdraw from the study.

E-mail invitations and reminders for follow-up questionnaires are registered in the local REDCAP database and sent automatically through the REDCAP secured platform, with access rights to the e-mail addresses strictly restricted to the investigator. A number of 3 reminders is automatically sent for completion by participants within the 30 days following the first invitation (approximately at day 10, 20 and 30).

Online questionnaires are implemented on the web, patients access it through a navigator (MS Edge, Firefox, Opera, Safari… for computers or phones). It is adapted for patients using computers, tablets or phones.

8.4 Collection of data for patients included in other trials assessing QoL

Two ongoing SIOP trials are based on a similar design than Harmonic, collecting quality of life data over 5 years after diagnosis of patients aged **5 years old or more at diagnosis**.

- **PNET5 (NCT:02066220)**
- **Ependymoma II (EP-II, NCT: 02265770)**

The investigators should check the subject's inclusion in one of these 2 trials to be sure that families agree to participate to the HARMONIC study while knowing that they might have to answer similar questions:

- Kindly warn the parents that participating to the HARMONIC study will involve answering similar but shorter questionnaires than for these trials (10 minutes by participant are required for the HARMONIC study).
- Report inclusion in PNET5 or Ependymoma II trials in REDCAP.

To enable or stop the sending of online questionnaires, here are the data that should be entered:

9.1 Enable questionnaires

BL - Demo&SocioEco

- **Month and date of birth** in BL - Demo&SocioEco. The age (dynamic age) will be calculated automatically.

BL&FU - H2.6 (QoL & Educ) form

The form includes 4 parameters as described below:

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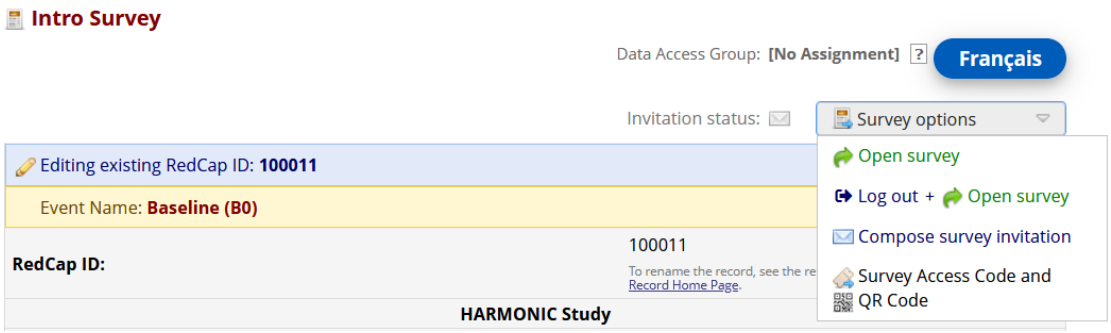
Which version of questionnaires was sent / will be sent to the patient or her/his parents/guardians?	◯ Paper-based questionnaires ◯ Electronic online questionnaires
Date of completion of the paper questionnaire	_____ (D-M-Y. Must provide the exact date)
End date of radiation therapy at baseline	_____ (D-M-Y. Must provide the exact date and be completed as soon as possible and ONLY at Baseline)
Email address of the patient or her/his parents/guardians	_____ (If online questionnaires are planned during follow-up, the email address must be provided and be updated if it is changed)

Each participating center have to inform their participants of the possibility to use paper-based or online version of questionnaires at Baseline visit.

9.2 For paper-based questionnaires

For each participant who use questionnaires in paper, please follow the 3 steps below to complete the patient's responses.

Step 1: Go to "Intro Survey" form at the concerning visit (e.g., at "Intro Survey" at Baseline or at M12…), Choose the corresponding language in the questionnaire (e.g., Français, Nederlands…), click at "Survey options" then "Open survey" to open the questionnaires according to the participant.



Step 2: Fill out the participant's responses.

If you do not have enough time to fill out all the patient's responses, you can click on the button Save and complete later.

Step 3: Once you complete all the participant's responses, please go to "BL&FU - H2.6 (QoL & Educ)" form at the concerning visit (e.g., at Baseline for baseline paper-questionnaires, at M12 for M12 paper-questionnaires).

Finally, click on **Paper-based questionnaires** and complete **Date of completion of the questionnaire**.

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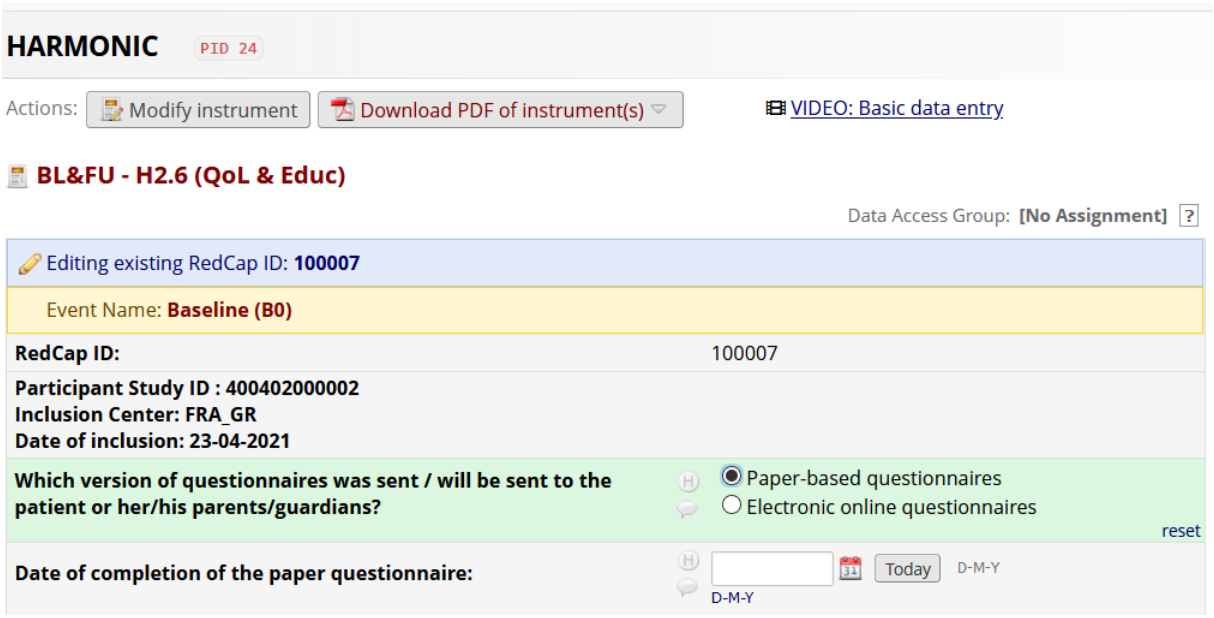
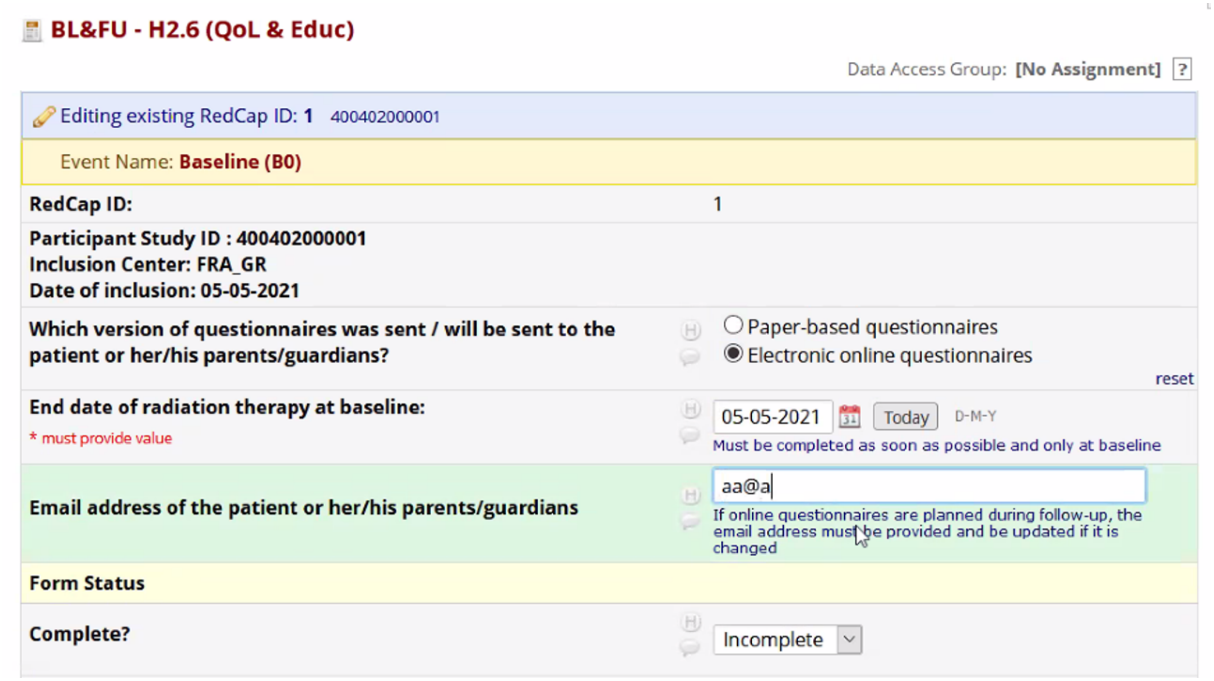


9.3 For electronic-online questionnaires

For each participant using online questionnaires, please go to "BL&FU - H2.6 (QoL & Educ)" form at the concerning visit (e.g., at Baseline for baseline questionnaires, at M12 for M12-questionnaire).

Click on **Electronic online questionnaires** and then, complete **Email address of the patient or her/his parents/guardians**. The email address will be automatically registered in the "Participant list" (see below the section "Sending of invitation at Baseline visit") in "Survey distribution tools" on the database and only your center's users can have right to access to.



For participants who do not change email address during study, you need to enter email address only once in "BL&FU - H2.6 (QoL & Educ)" at Baseline as the email address will be registered in the database system.

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For participants who change email address during study, please enter the new email address as soon as possible in **Email address of the patient or her/his parents/guardians** at the corresponding visit. The old ones will be replaced automatically by the new ones in the database system. (e.g., go to M36 for address emails change before M36 in order that the participants can receive invitations in their new email box from M36.

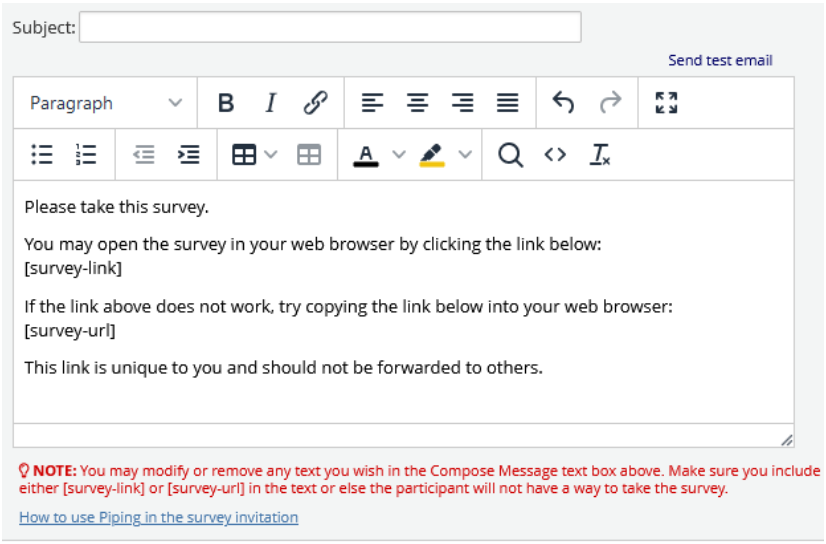
If you use a local database, you have to complete **End date of radiation therapy at baseline** as soon as possible to benefit from automated invitations already scheduled for all visits during follow-up from M12 to M120. Furthermore, beware when you export data towards the central database: you will have to uncheck participants' email addresses while uploading data to HARMONIC central database in the Inserm server.

Email template preparation

All participants who use online questionnaires will receive an email with indications on how to answer to the questionnaires. It is important to let contact information of investigator(s) in this email in order that the participants can ask questions concerning the questionnaire and/or the study if necessary.

In HARMONIC database, there is a default email in English **that has to be changed and translated for each center** following the email Template for HARMONIC participants. Beware, you must leave the characters [survey link] and [survey-url] **exactly as they are** to enable redcap to insert the personalized link for each patient as indicate in red below the default email.

The default email is as follows:



The email template for the HARMONIC study is as follows:

Subject: Questionnaire for HARMONIC participants

Dear HARMONIC participant, Thank you for participating to the HARMONIC study, which evaluates the long-term effects of cancer treatment on quality of life. The information reported by patients and their families on quality of life is very important to properly monitor patients' health and adapt future treatments. Beyond your doctor's advice and medical information, it is very important to know your perception and your personal experience.

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This is why we kindly ask you today to fill a questionnaire by clicking on the link below. It only takes **about 10 minutes to complete**.

Remember that your answers are confidential: the researchers of the HARMONIC study are the only ones to have access to them. The study fulfils all legal requirements to guarantee the protection of your data.

Please click on the link below to open the questionnaire and participate:
[survey-link]

If the link above does not work, please try to copy the link below into your web browser to open the questionnaire:
[survey-url]

This questionnaire is unique to you and should not be forwarded to others.

If you have any question concerning the questionnaire or the study, please do not hesitate to contact the HARMONIC team by replying to this email.

Thank you very much for your participation!

The HARMONIC team

If you use HARMONIC central database in Inserm server

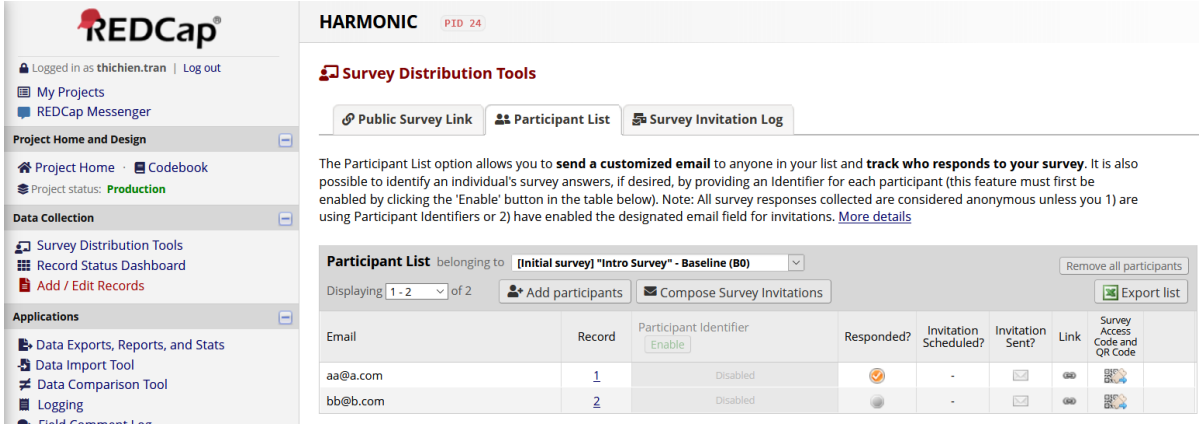
To be able to send online questionnaires to participant(s) from Baseline, please follow the 4 steps below:

Step 1: Indicate the concerning visit

Please go to “Survey distribution tools” / “Participant list” at **“**belonging to”, please choose the visit that you want to send online questionnaires:

- **[Initial survey] “Intro Survey” – Baseline (B0)**
- Or **“Intro Survey” – M0**
- Or **“Intro Survey” – M12**
- Or **“Intro Survey” – M36**
- Or **“Intro Survey” – M60**
- Or **“Intro Survey” – M120**

And then click on “Compose Survey Invitations” to prepare invitations at the chosen visit.



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You can see email addresses of the participant and the others according to the record number in your center that you have registered as indicated in the previous section (screenshot above in the section “For electronic-online questionnaires”).

Step 2: Schedule invitations

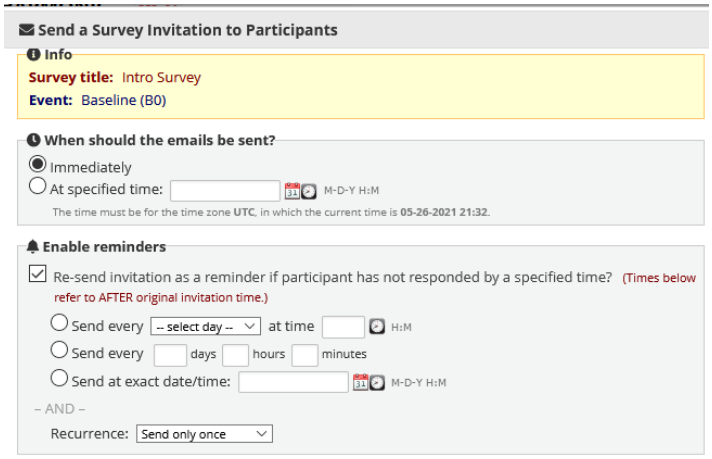
For Baseline

You can choose the date of sending (“Immediately” or “At specified time”) and the reminders but you must ensure that participant(s) answer questionnaires **before receiving the first dose of EBRT**.

For the other visits

As soon as you get **End date of radiation therapy at baseline** of participant(s), you have to schedule automated invitations and reminders not only for M0 but also for M12, M36, M60 and M120 in order that online questionnaires will be automatically sent to each of them at every visits from M0 until the end of the study or the **Date of Withdrawal** (see condition for withdrawal in the section 4 below). To do that, please enter “At specified time” the date of the visit that you have chosen in **Step 1**.

- If you choose “**Intro Survey” – M0**, date of M0 is a date immediately after the last fraction of EBRT at Baseline, or within 3 months after completion of EBRT at baseline
- If you choose “**Intro Survey” – M12**, date of M12 = M0 + 12 months
- If you choose “**Intro Survey” – M36**, date of M36 = M0 + 36 months
- If you choose “**Intro Survey” – M60**, date of M60 = M0 + 60 months
- If you choose “**Intro Survey” – M120**, date of M120 = M0 + 120 months.



Please avoid sending emails to patients during the weekends. Tuesdays-Fridays at 5:00 PM may be an appropriate timing for instance.

Step 3: Choose the sender and compose the invitation email by typing on the case “From” and choosing a user’s address from drop list (e.g., louhen@rm.dk).

The sender(s) need to be investigator(s) or her/his representative in your center so that the participants know whom to ask questions concerning the questionnaire or the study by replying to the invitation email.

Enter the invitation email’s subject and then compose the message (see above the template).

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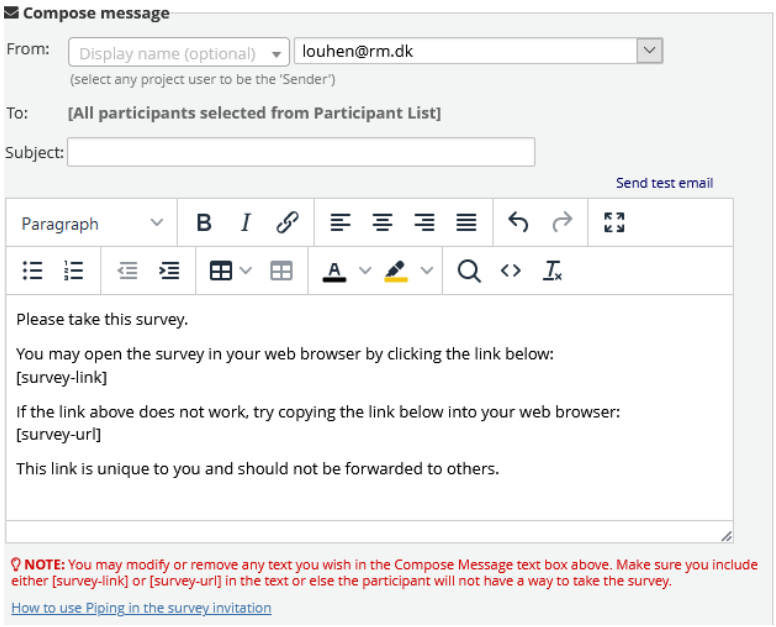


Step 4: Choose participation(s) to send invitations

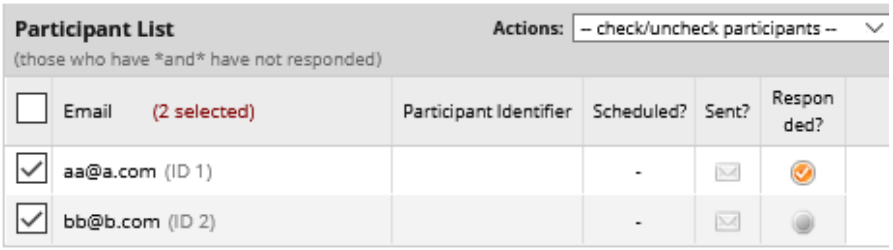
Among the participants' email addresses, select only one(s) that you want to send to.

If you send invitations to only one participant (e.g., record 1), please click only on the participant's email address (aa@a.com).

If you send invitations to a group of participants (participants with **End date of radiation therapy at baseline** is found in a same month. e.g., record 1 and record 2), please click on all their email addresses (aa@a.com, bb@b.com).

Please avoid scheduling invitations to participants included in different months.

To finish this step, please click on "Send invitations" at the end of the page.



If you use HARMONIC local database based in the server of your center

For sending online questionnaires at Baseline or M0

To be able to send online questionnaires to participant(s) at Baseline or M0, please follow the 4 steps as described in the above section "If you use HARMONIC central database in Inserm server".

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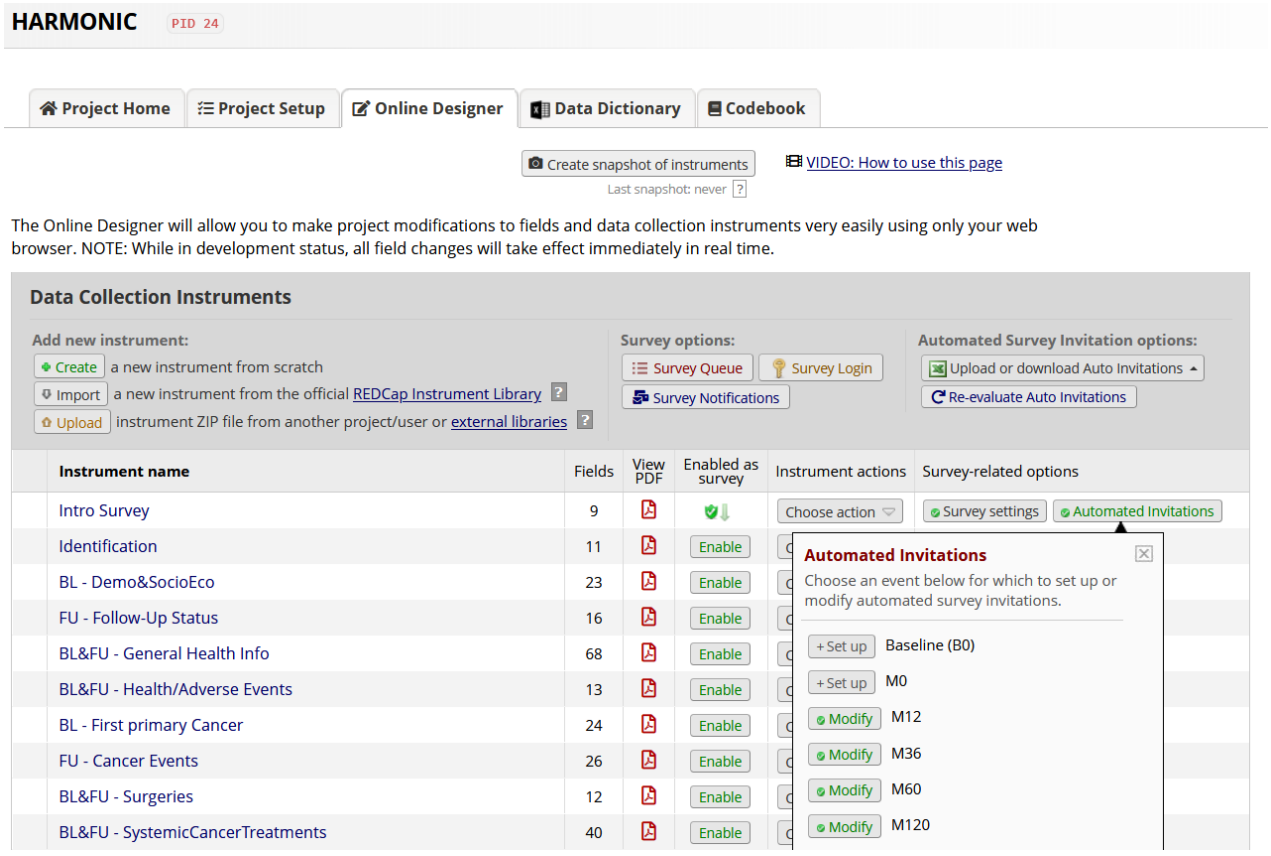
For sending online questionnaires from M12

As soon as you can after receiving HARMONIC database and before moving it in "Production" status, you have to replace the default message by the email Template (see above the template). You also need to modify conditions for sending if necessary at each of the visits M12, M36, M60 and M120. That means you re-schedule automated invitations for your local database. Consequently each of all participants whose email addresses have been registered on the database will receive by default a common invitation and her/his personal questionnaire by email(s) at each of her/his visit during follow-up from M12 to M120 or until the **Date of Withdrawal** (see condition for withdrawal in the section 4 below).

Please re-schedule invitations for each of the visits M12, M36, M60 and M120 by following 3 steps below:

Step 1: Choose a visit during follow-up

Please go to « Designer » / « Automated Invitations » and click on "Modify" at a visit during follow-up (e.g., "Modify" M12).



Step 2: Define condition for automated online questionnaires

At the chosen visit (e.g., M12) click on "Automated Invitations".

In "Define condition for automated online survey", you can see the default message. Please replace it by the email Template (see section "Email template preparation").

The sender will be the administrator of the local database. If you want other persons received questions from participants, please indicate them in your email Template.

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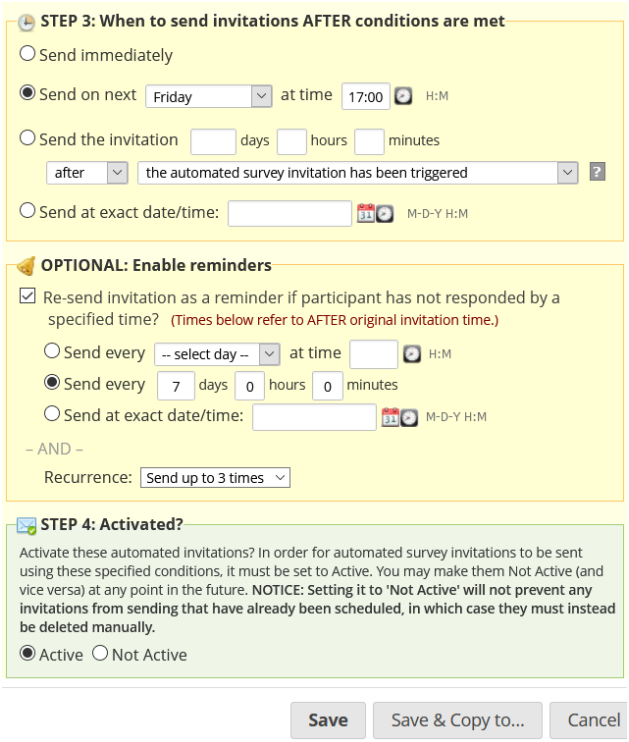
Step 3: Modify conditions for sending online questionnaires if necessary

Please go to “Step 3: When to send invitations AFTER conditions are met” to modify the date of sending if necessary (e.g., send immediately at the date of M12 instead of Friday after M12).

And then go to “OPTIONAL: Enable reminder to modify the dates for sending email reminders if necessary (e.g., reminders every 7 days instead of 15 days after the automated survey invitation has been triggered).

Finally, click on “Save” to finish scheduling automated invitations.

In any case, please avoid sending emails to patients during the weekends. Tuesdays-Fridays at 5:00 PM may be an appropriate timing for instance.



Participant response status

From “Participant List”, you can check response status of each participant

- If the bouton “Responded” is green, the participant has completed the questionnaire
- If the bouton “Responded” is orange, the participant has opened the questionnaire but has not completed it, yet.
- If the bouton “Responded” is gray, the participant has not opened the questionnaire, yet.

The email reminder will be stopped automatically after completing the questionnaires.

Please check response status of participants to help them to answer questionnaires in the given time of each visit.

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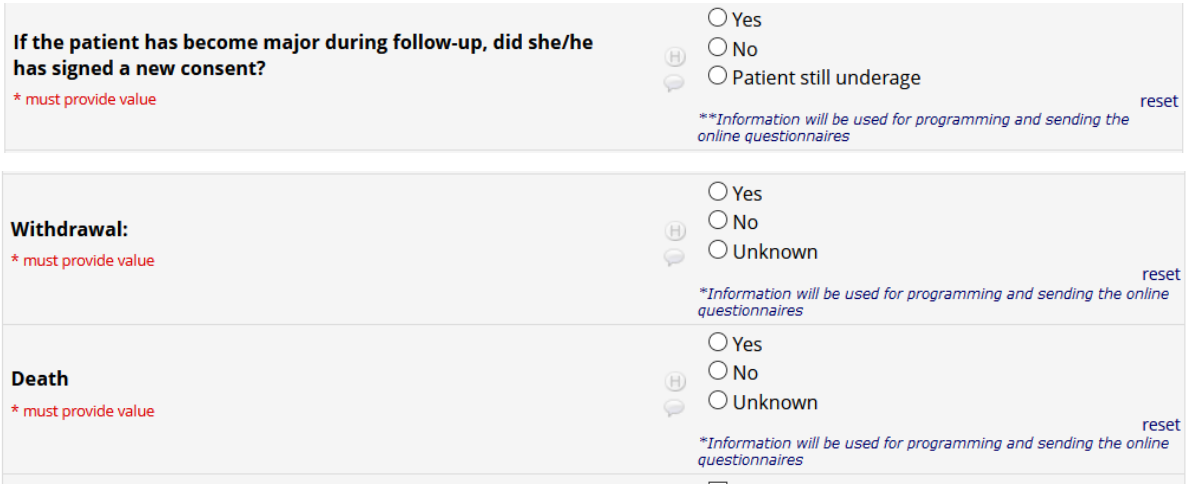
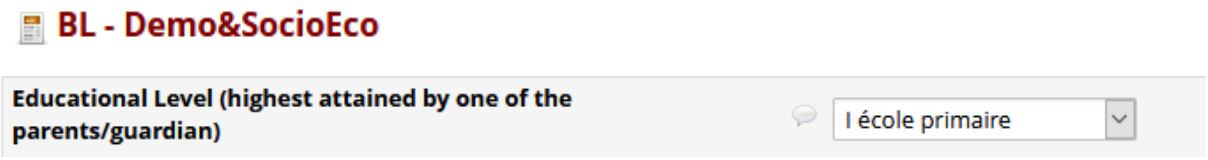
9.4 Stop the questionnaire: Withdrawal (death, consent, etc.)

Information on the new consent for patients who have become major during follow-up, Withdrawal from the study has to be recorded in FU - Follow-Up Status on REDCAP as soon as possible to stop the sending of the questionnaire.



9.5 Non-response to the baseline questionnaire

In case of **non-response to the baseline questionnaire**, kindly ask the mother or the father to report his/her educational level and enter it into REDCAP (BL – Demo&Socioeco).



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- **At inclusion**
    3. Propose paper-based questionnaires primarily. The parent and the child should complete the questionnaires independently of one another, if feasible.
    4. Check inclusion of the patient in the **SIOP** trials. Kindly warn the parents that some questionnaires might be similar between these trials and Harmonic. Report inclusion in these trials, whether the family accepts or refuse to participate in the QoL task of Harmonic.
    5. Enter the **appropriate data – month and date of birth, Date of end of radiation therapy (when known), email address – to enable** the sending of the online questionnaires.
    6. In case of **non-response to the baseline questionnaire**, kindly ask the mother or the father to report his/her educational level and enter it into REDCAP.
    7. Enter manually the data of the paper-based questionnaires, including date of completion.

- When the questionnaires have not been completed during the clinic visit for any reason (except study withdrawal), online questionnaires can be proposed.

- **Enter the date of M0 (Date of end of radiation therapy) as soon as you know it**. Activate the programming of the online questionnaires in REDCAP.

- **When the patient reaches 18 years old**, collect his/her email address when making him/her sign the new consent form, to send the online questionnaire directly to him.

- **Follow-up: immediately** report any information on **withdrawal** to stop automated sending of the questionnaires.

- Data entered by the investigators are collected into the local REDCAP database. Data stored locally are regularly exported towards the core database (every six months).

If there is any doubt or question concerning the procedures described in this manual, please feel free to contact the HARMONIC-QOL referees: agnes.dumas@inserm.fr ; Thichien.TRAN@gustaveroussy.fr

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