CLINICAL PROTOCOL

ULTIMO

Protocol no CTO21030GZA

Version 3.0 20-MAY-2025

ZAS vzw

Kempenstraat 100
2030 Antwerpen

Trial title, protocol version and registration

English title: Ultrahypofractionated versus normofractionated sequential boost after whole-breast radiation therapy in patients treated with breast-conserving surgery for breast cancer (ULTIMO)

Dutch title: Ultragehypofractioneerde, versus normaalgefractioneerde sequentiële boost na volledige borst radiotherapie, in patiënten na borst-sparende heelkunde voor borstkanker (ULTIMO)

Current protocol: Version 3 - 20-MAY-2025

Trial registration

Registry	Unique Id	Date of first registration
Local trial registry (central IEC)	CTO21030GZA	17-FEB-2022

Statement of compliance

This study will be conducted in compliance with this clinical study protocol, the current International Conference on Harmonization and the guidelines for Good Clinical Practices (ICH-GCP), the principles of the Declaration of Helsinki (version 2024) and any applicable regulatory requirements. Enrolment at any clinical study site may not begin prior to that site receiving approval from the ethics committee of record for the protocol and all materials provided to potential subjects.

Any amendments to the protocol or changes to the consent document will be approved before implementation of that amendment. Reconsent of previously enrolled subjects may be necessary depending on the nature of the amendment.

The Principal Investigator will ensure that changes to the study plan as defined by this protocol will not be made without prior agreement from the Sponsor and documented approval from the ethics committee of record, unless such a change is necessary to eliminate an immediate hazard to the study subjects.

All personnel involved in the conduct of this study have completed Human Subjects Protection and GCP Training as outlined by their governing institution.

Confidentiality Statement

Roles and responsibilities

Coordinating team and centre (ZAS Augustinus)

Prof. Dr. Melanie Machiels, Radiation oncologist in ZAS – Iridium Netwerk[1]	Principal Investigator (PI), involved in Protocol development and trial management[a]
Dr. Tom Quisenaerts, Plastic-Reconstructive surgery resident and PhD-candidate[1]	Sub-investigator (SI), Project manager, involved in Protocol development and trial management[a]
Prof. Dr. Philip Poortmans, Radiation oncologist in ZAS – Iridium Netwerk[1]	Sub-investigator (SI), involved in Protocol development and trial oversight
Ilse Van Der Auwera, Project manager ZAS CTO[1]	Project manager, involved in Protocol development and trial management[a]

Trial sponsor and funding	
Ziekenhuis aan de Stroom (ZAS) vzw, represented by CEO **Willeke Dijkhoffz**[2]	Sponsor, delegates trial responsibilities to the PI and their delegates
Iridium netwerk vzw, represented by Philippe Huget[3]	Funder, delegates trial responsibilities to the PI and their delegates. Requests annual updates in order to assess correct trial conduct and viability.

Trial writing committee	
Prof. Dr. Melanie Machiels, Radiation oncologist in ZAS – Iridium Netwerk[1]	Writing of the study report and scientific or public disseminations
Dr. Tom Quisenaerts, Plastic-reconstructive surgery resident and PhD-candidate[1]	Writing of the study report and scientific or public disseminations

[a]: Trial management will include overseeing data collection and handling, data analysis (supported by a statistician consultant), data interpretation.

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Funding

This clinical trial is financially funded by “Iridium netwerk vzw”, providing a grant covering all direct study related expenses. This is an organisation of radiotherapists based in Antwerp, promoting radiotherapy focussed research, among other activities.

The indirect/overhead costs such as the functioning of the ZAS Augustinus clinical trial centre, and additional (unforeseen) costs will be covered by “Iridium network vzw” and “Ziekenhuis aan de Stroom vzw” (ZAS).

Sponsor's Approval

Protocol title: Ultrahypofractionated versus normofractionated sequential boost after whole-breast radiation therapy in patients treated with breast-conserving surgery for breast cancer (ULTIMO trial).

Version number and date: 3.0 20-MAY-2025

The design of this study as outlined by this protocol has been reviewed and approved by the Sponsor's responsible personnel and principal investigator, as indicated below.

Sponsor Representative:

Signature: _____ Date: _____

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Study responsible physician:

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Name: Prof. Dr. Melanie Machiels

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Investigator Agreement

Protocol title: Ultrahypofractionated versus normofractionated sequential boost after whole-breast radiation therapy in patients treated with breast-conserving surgery for breast cancer (ULTIMO trial).

Version number and date: 3.0 20-MAY-2025

I have read the protocol, appendices, and accessory materials related to the "*Ultrahypofractionated versus normofractionated sequential boost after whole-breast radiation therapy in patients treated with breast-conserving surgery for breast cancer (ULTIMO trial).*" clinical trial and agree to the following:

- To conduct this study as described by the protocol and any accessory materials
- To protect the rights, safety, and welfare of the subjects under my care
- To provide oversight to all personnel to whom study activities have been delegated
- To conduct the study in accordance with all applicable local and national regulations, the requirements of the ethics committee of record for my clinical site, and Good Clinical Practices as outlined by ICH GCP E6(R2)
- To obtain approval for the protocol and all written materials provided to subjects prior to initiating the study at my site
- To obtain informed consent – and updated consent in the event of new information or amendments – from all subjects enrolled at my study site prior to initiating any study specific procedures or administering investigational products to those subjects
- To maintain records of each subject's participation and all data required by the protocol

Signature: _____ Date: _____

Name: _____

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List of abbreviations

Abbreviation	Definition
AE	adverse event
AIS	Aesthetic Items Scale
BCT	breast-conserving therapy
BCS	breast-conserving surgery
CBCT	Cone-beam computed tomography
CT	Computed Tomography (scan)
CTCAE	common terminology criteria for adverse events
CTO	Clinical Trials Office
DCIS	Ductal Carcinoma In Situ
EC	Ethics Commission
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EORTC	European Organisation For Research And Treatment Of Cancer
EoS	End of Study
ESTRO	European Society for Radiotherapy and Oncology
FF	FAST forward
FU	Follow-Up
Gy	Gray (SI unit)
HER2	Human Epidermal growth factor Receptor 2
ICF	informed consent form
ICH-GCP	current International Conference on Harmonization and the guidelines for Good Clinical Practices
IEC	independent ethics committee
IQR	Inter-Quartile Range
IRB	Institutional Review Board
LST	Last Study Treatment
MeV	Mega-electro Volt
MRI	Magnetic Resonance Imaging
MV	Mega Volt
normSEB	Normofractionated sequential boost
OAR	Organs At Risk
OS	Overall survival
pCR	pathologic Complete Remission
PET	Positron Emisison Tomography (scan)
QoL	Quality of Life
RT	Radiation Therapy
Rz	Randomisation
RS	Raw Score
S	Scale score
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SoC	Standard of Care (treatment)
SoE	Schedule of Events
SUSAR	Suspected unexpected serious adverse reaction
TAS	Total Aesthetic Score
UltSEB	Ultrahypofractionated sequential boost
WBRT	whole breast RT
ZAS	Ziekenhuis Aan de Stroom

Amendments

History of the protocol and its amendments	
Protocol version	**Date of IEC application**
Protocol v1.0, original protocol	17-FEB-2022
Protocol v2.0, amendment	20-SEP-2022
Protocol v3.0, amendment	Current review 20-MAY-2025

Protocol v3.0, amendment 20-MAY-2025

Purpose of this amendment:

1. Amendment to the sample size calculation.
2. Addition of a second primary outcome to improve the expected power after sample size amendment.
3. Operationalisation of the outcome variables, including detailed description.
4. Definition of all collected data.
5. Improved definition of the statistical methodology.
6. Overhaul of general aspects of the protocol, to improve readability and clarity.
7. Removal of secondary outcome “Health economic impact”.

A summarized list of key changes is provided in Appendix 9: amendment history

Synopsis

Title	Ultrahypofractionated versus normofractionated sequential boost after whole-breast radiation therapy in patients treated with breast-conserving surgery for breast cancer (ULTIMO trial).
Protocol number	CTO21030GZA
Secondary identifier(s)	BUN B2022099000001
Study sites	Monocentric, ZAS Augustinus (GZA, St.-Augustinus)
Disease under study	Breast cancer
Study Objectives and Endpoints	
Objective	**Endpoint**
Primary	
Cosmetic non-inferiority (BCCT.core)	**Operationalisation (measurement variable):** BCCT.core is a computer vision software packages, which uses photos of the breasts as input, taken as described in Section 8.7, and Appendix 5. The output comprises of 10 variables relating to how to software scores breast cosmesis. The 'Global cosmetic result' from the three photos will be averaged and then used as the individual value of this measure. The results are scored on a 4-point Likert scale, coded as 0-3. Only the two frontal photographs (arms up and down) will be used, and their score will be averaged to produce the outcome measure. **Analysis metric:** The change from baseline at 3 years of follow-up will be used. Negative values will be transformed to '1' to signal an inferiority event, positive or values of zero will be transformed to '0' to signal non-inferiority. **Method of aggregation:** The results will be reported as a contingency table reporting both frequencies and proportions of non-inferior and inferior results in each treatment arm. **Time point(s):** A baseline assessment is performed during the screening visit. The primary endpoint is assessed at 3 year of follow-up after the last study treatment (LST). **Rationale:** This study was designed as a non-inferiority trial. The BCCT.core software offers an objective assessment of aesthetic outcome. By dichotomising the results, they become easier to interpret and compare to the existing literature, however this implies a trade-off in power/sample size.
Cosmetic outcome (Expert panel; AIS-TAS & AIS-Symmetry)	**Operationalisation (measurement variable):** The AIS is a tool for scoring breast cosmesis based on standardized photos. It comprises of 5 items, each comprising of a 5 point Likert-scale, coded as 1 to 5. The score is given based on the group of photos taken at a single time point. The scores of all items are summed to produce the TAS, which consequently ranges from 5 to 25. The AIS is used in an expert panel setting, where all scores per item are averaged and then used as the individual value of this measure. More details can be found in Section 8.7, and Appendix 7. **Analysis metric:** AIS-Symmetry and AIS-TAS absolute values will be used for analysis.

	Method of aggregation: The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. The primary endpoint is assessed at 3 year of follow-up after the last study treatment (LST). **Rationale:** As discussed in the sample size amendment, this outcome was introduced in order to move away from the dichotomous outcome, and introduce a continuous variable in order to improve the power of this study, for the (second) primary outcome. The AIS-tool was selected due to its simplicity and good inter-rater validity in professionals.
Secondary	
Cosmetic non-inferiority (BCCT.core)	**Operationalisation (measurement variable):** Cfr. supra (BCCT.core primary outcome) **Analysis metric:** Cfr. supra (BCCT.core primary outcome) **Method of aggregation:** Cfr. supra (BCCT.core primary outcome) **Time point(s):** A baseline assessment is performed during the screening visit. The secondary endpoint is assessed at 1 year of follow-up after the last study treatment (LST). **Rationale:** Cfr. supra (BCCT.core primary outcome)
Cosmetic outcome (Physician scoring)	**Operationalisation (measurement variable):** The physician scoring scale (Section 8.7, and Appendix 2) will be used to assess cosmetic outcome. The normalised average score of all items will be summed and used as outcome measure. This will be calculated as the score of an item minus one, divided by the amount of answering options minus 1 (k-1), averaged over all items. **Analysis metric:** Absolute values will be used for analysis. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. It is then assessed again at 1 year, and 3 years of follow-up after the last study treatment (LST). **Rationale:** This scale offers a concise evaluation of important and relevant items in the assessment of post BCS+WBRT treatment.
Cosmetic outcome (Patient scoring; PROM)	**Operationalisation (measurement variable):** The cosmetic evaluation questionnaire by Sneeuw at al. (Section 8.7, and Appendix 1; Section) will be used to assess cosmetic outcome. The normalised average score of all items will be summed and used as outcome measure. This will be calculated as the score of an item

	minus one, divided by the amount of answering options minus 1 (k-1), averaged over all items. **Analysis metric:** Absolute values will be used for analysis. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. It is then assessed again at 1 year, and 3 years of follow-up after the last study treatment (LST). **Rationale:** This scale is a validated tool for cosmetic assessment.
Quality of life (QLQ – C30 v3)	**Operationalisation (measurement variable):** The QoL (QLQ-C30) outcome variable is operationalised through the QLQ-C30 questionnaire, included in Appendix 3 and discussed in Section 8.8. The answers are transformed into 3 separate scores, which can all range from 0 to 100. These scores are: Global health status/QoL; Functional scales; Symptom scales. Where a higher score on the first 2 scales indicate a better health state and QoL, while on the symptom scale a higher score indicates a higher level of disturbance by symptoms. **Analysis metric:** The QLQ-C30 Global health status/QoL; Functional scales; Symptom scales, absolute values will be used for analysis. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. It is then assessed again at 1 year, and 3 years of follow-up after the last study treatment (LST). **Rationale:** The QLQ-C30 questionnaire offers a validated questionnaire assessing QoL.
Quality of life (QLQ – BR23 v1)	**Operationalisation (measurement variable):** The QoL (QLQ-BR23) outcome variable is operationalised through the QLQ-BR23 questionnaire, included in Appendix 4 and discussed in Section 8.8. The answers are transformed into 2 separate scores, which can both range from 0 to 100. These scores are: Functional scales; Symptom scales. Where a higher score on the functional scale indicate a better health state and QoL, while on the symptom scale a higher score indicates a higher level of disturbance by symptoms. **Analysis metric:** The QLQ-BR23 Functional scales; Symptom scales, absolute values will be used for analysis. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):**

	A baseline assessment is performed during the screening visit. It is then assessed again at 1 year, and 3 years of follow-up after the last study treatment (LST). **Rationale:** The QLQ-BR23 questionnaire, as a subset of the QLQC30 offers a validated questionnaire assessing QoL, specifically pertaining to breast cancer patients.
Local recurrence free survival, and overall survival:	**Operationalisation (measurement variable):** The local recurrence free survival outcome variable is operationalized as recurrence free survival, measured as time to event for local recurrence. The overall survival outcome is operationalized as time to death, paired with disease related death 'yes/no'. As discussed in Section 8.10. **Analysis metric:** The time to event data is registered in days from end of treatment. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** Local recurrence free, and overall survival will be reported at 1, and 3 years of follow-up after the last study treatment (LST). **Rationale:** In order to assess oncological safety of the novel treatment, the local recurrence will be assessed as a secondary outcome variable. The main goal is to observe whether there is a difference, in order to make sure that there is no increase, while also assessing any potential improvement.
Fibrosis	**Operationalisation (measurement variable):** During the study all adverse events (AEs) are monitored and collected in the eCRF, based on the 'National Cancer Institute Common Terminology Criteria for Adverse Events' (NCI-CTCAE) v5.0 reporting system. The AE codes and grades will be recorded in the eCRF and used as variables. The NCI-CTCAE v5.0 and (S)AE registration is discussed in Section 8.9, Section 9, and Appendix 6. For this outcome measure 'Fibrosis' will be tabulated. **Analysis metric:** Tabulation of absence vs. presence and severity will be used for analysis. **Method of aggregation:** The proportion of participants experiencing no 'Fibrosis'-AE vs. any 'Fibrosis'-AE will be used. As well as the frequency of all grades, including 0 (absence). **Time point(s):** AEs will be assessed and recorded continuously, with explicit querying during all follow-up visits. These outcome variables will be reported during interim- and final analyses. **Rationale:** Fibrosis is one of the main radiotherapy comorbidities after BCT, with a significant impact on both cosmetic and breast satisfaction outcomes.

Breast pain	**Operationalisation (measurement variable):** During the study all adverse events (AEs) are monitored and collected in the eCRF, based on the 'National Cancer Institute Common Terminology Criteria for Adverse Events' (NCI-CTCAE) v5.0 reporting system. The AE codes and grades will be recorded in the eCRF and used as variables. The NCI-CTCAE v5.0 and (S)AE registration is discussed in Section 8.9, Section 9, and Appendix 6. For this outcome measure 'Breast pain' will be tabulated. **Analysis metric:** Tabulation of absence vs. presence and severity will be used for analysis. **Method of aggregation:** The proportion of participants experiencing no 'Breast pain'-AE vs. any 'Breast pain'-AE will be used. As well as the frequency of all grades, including 0 (absence). **Time point(s):** AEs will be assessed and recorded continuously, with explicit querying during all follow-up visits. These outcome variables will be reported during interim- and final analyses. **Rationale:** Pain in the treated breast is one of the main radiotherapy comorbidities after BCT, with a significant impact on both cosmetic and breast satisfaction outcomes.
Study Design	
Study design synopsis	The ULTIMO study is a monocentric, prospective, randomised controlled, non-blinded, phase III interventional clinical trial, with a non-inferiority design, in patients with breast cancer undergoing breast conservative treatment (BCT). After giving informed consent and verifying eligibility, data collection starts and patients will be randomized in one of the following treatment arms: - **Standard treatment arm:** Breast Conserving Surgery (BCS) takes place, followed by the SoC hypofractioned WBRT and SoC normofractioned sequential boost (normSEB) of 10Gy over 5 fractions. - **Experimental treatment arm:** Breast Conserving Surgery (BCS) takes place, followed by the SoC hypofractioned WBRT and experimental ultrahypofractionated sequential boost (ultSEB) of 6Gy in a single application. The primary objective of the ULTIMO study is to assess whether the ultSEB RT boost protocol is non-inferior to the current SoC normSEB RT boost protocol. This assessment will be based on the cosmetic outcome of the breasts, while also taking into account local recurrence rates and the frequency and intensity of (S)AEs.
Sample size	n=132
Eligibility criteria	All women with breast cancer referred for postoperative WBRT with an indication for a boost to the lumpectomy cavity according to institute guidelines will be eligible. **Inclusion criteria** - Breast cancer patients referred for WBRT + boost to the lumpectomy cavity - Patients ≥18y - Karnofsky Performance Score >70%, or ECOG <2 - Life expectancy of more than 5 years

	- Invasive tumour free resection margins, defined as R0 resection on pathology report - Written informed consent **Exclusion criteria** - Previous contralateral breast cancer - Previous RT of the same breast or thorax. - Metastatic disease (M1)
Length of Participation	Follow-up visits to assess safety and efficacy will occur as delineated in the Schedule of Events (SOE). The first follow-up visit occurs at 1 year after last study treatment (LST). The second, and final follow-up visit, assessing the primary endpoint will take place at 3 year after LST. After reaching the primary endpoint, participant follow-up will be concluded.
Intervention	Patients are randomised to control arm or experimental arm, respectively receiving 5 or 1 daily fractions of 2 or 6Gy each, to the lumpectomy cavity / tumour bed. Preferably, the overall treatment time is 1 week of irradiation in the normSEB group. We estimated 6 Gy in 1 fraction to the tumour bed to be equivalent to the normSEB which is traditionally 10 Gy in 5 fractions. We do not expect increased early toxicity rates since biological equivalent dose remains the same as with normofractionation (1). The boost dose is given sequential without any gaps after WBRT. For the WBRT the target area encompasses the complete glandular breast tissue and – if indicated – the lymph node regions according to current ESTRO guidelines. Organs at risk (OAR) are contoured, comprising both breasts, both lungs, the heart, the thoracic wall (ribs and musculature), and the skin.
Statistical Methods	The sample size was calculated based on the proportion of inferior cosmetic outcome after WBRT+Boost, known from the literature to be 20%. A non-inferiority sample size calculation was performed for delta = 10%, power = 80%, and alpha = 5%. This resulted in n=108, accounting for up to 20% dropout, this was elevated to n=132. An amendment to this sample size calculation was later added, cfr. Section 10.1. The new power analysis based on the newly introduced second primary outcome, starting from the number of participants included in this study states that for n=110, treatment mean = 96%, and Delta = 15%, the calculated power is 84.57%, or 97.65% when both means are assumed to be equal. The primary outcome will be evaluated using the Farrington-Manning test for non-inferiority between proportions will be used, cfr. Section 10.3. The second primary outcome will be analysed using a non-inferiority adapted t-test, for two independent continuous samples. Secondary outcomes will be evaluated using the Mann-Whitney U test to determine if there is a statistically significant difference between the control and experimental arms, cfr. Section 10.3. Statistical methods will be further outlined in a Statistical Analysis Plan (SAP).

Schedule of Events

Table 1. Schedule of events for patients in

Period	Ref.	Screening	Treatment period	Follow-up Period		Closeout
Visits		**Screening visit**	**WBRT**	**1 Year FU visit**	**3 Year FU visit**	**Closeout visit [g]**
Study Visit number		1	-	2	3	
Scheduling		≤10 bd of ICF		LST +1Y	LST +3Y	
Window				+/-1M	+/-1M	
ICF [a]	11.2	Before visit				
Enrolment						
Eligibility screen	4.	x				
Demographics	8.1	x				
Health data	8.2	x				
Concomitant medications	8.2	x				
Karnofsky/ECOG score	8.2	x				
Randomisation(RZ) [b]	7.2	x				
Assessments (baseline, treatment and follow-up)						
Cosmesis - Physician scoring	8.7	x		x	x	(x)
Cosmesis - patient scoring	8.7	x		x	x	(x)
QoL questionnaire - BR23 v1	8.8	x		x	x	(x)
QoL questionnaire - C40 v3	8.8	x		x	x	(x)
Fibrosis and breast pain	8.9			x	x	(x)
Photographs	8.7	x		x	x	(x)
AE assessment [c]	9.			x	x	(x)
Data on systemic therapy, RT and surgery	8.3, 8.4, 8.5		x			
Data on pathology and pathological tumour response	8.6			x	x	(x)
Data on oncological survival [d]	8.10	x	x	x	x	(x)
Study related interventions/treatments						
Radiotherapy	8.3		x			

Abbreviations: Ref. = reference within this document; RT = Radiotherapy; WBRT = Whole Breast Radiotherapy; FU = Follow-Up; bd = business days; ICF = Informed Consent Form; Rz = Randomisation; LST = Last study treatment; M = month (30 days); Y = Year (365 days); ECOG = Eastern Cooperative Oncology Group; AE = Adverse Event; OS = Overall survival

a. Informed consent from the patient must be documented before any study specific procedure, including procedures for screening, are undertaken.
b. Patients can be randomized as soon as eligibility has been confirmed by the Coordinating Investigator.
c. Elicitation of all AEs will occur at each interaction with the patient from the time of informed consent. Patients will be questioned at each visit, regarding AEs and will also be instructed to inform the Investigator or clinic staff of any AEs or intercurrent illnesses experienced at any time during the trial. Adverse events will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (NCI-CTCAE v5.0).
d. Oncological follow-up of patients will be planned according to local institutional guidelines.
e. In the case of premature discontinuation from study participation, the subject should be asked to return to the clinical site and complete an early termination visit. The indicated assessments can be considered depending on the status of the participant, timing of discontinuation and the consent of the participant.

Note: additional unscheduled visits may occur at the discretion of the Investigator, i.e. if considered necessary for clinical safety reasons.

1. Background & Rationale

In breast conserving therapy (BCT), radiation therapy (RT), which follows breast-conserving surgery (BCS), is performed as whole breast RT (WBRT) followed by a boost dose to the lumpectomy cavity (i.e. the tumour bed) depending on the risk profile.

For WBRT, hypofractionation with 15 or 16 fractions instead of the classic 25 fractions is the current standard of care. Equal or less toxicity was seen with the hypofractionated schedules used in both the Canadian hypofractionation trial and the UK START trials (2,3). More recently, the FAST Forward schedule (5 fractions) was published and showed equivalent results for late toxicity, relapse and survival at 5 years after irradiation as a 15-fractions schedule of 2.67 Gy over 3 weeks, and has become the new standard of care for WBRT, significantly increasing quality of life of treated patients (4).

Nonetheless, when the patients` risk profile requires a boost dose, this is still administered sequentially in 5–8 fractions, which at least doubles the treatment time and increases the number of hospital visits, potentially negatively impacting quality of life. In Belgium, a boost dose is administered according to institute-dependent guidelines. However, this often includes all patients aged <70, resulting in vast numbers of patients requiring a boost radiation therapy dose.

The question arises, whether the boost given to the lumpectomy cavity could also be given in an **ultrahypofractionated scheme** (i.e. in one single fraction). An ultrahypofractionated sequential boost (ultSEB) technique may lead to a number of therapeutic advantages including: (1) radiobiological exploitation of a lower alpha/beta ratio of breast cancer tumour cells by delivering a larger fractional dose to the at-risk region, (2) logistical ease for patients by shortening overall treatment length, and (3) economic benefits by improving patient-related costs, as it enables treatment of a higher number of patients over shorter time intervals.

2. Study objectives and endpoints

Study Objectives and Endpoints	
Objective	**Endpoint**
Primary	
Cosmetic non-inferiority (BCCT.core)	**Operationalisation (measurement variable):** BCCT.core is a computer vision software packages, which uses photos of the breasts as input, taken as described in Section 8.7, and Appendix 5. The output comprises of 10 variables relating to how to software scores breast cosmesis. The 'Global cosmetic result' from the three photos will be averaged and then used as the individual value of this measure. The results are scored on a 4-point Likert scale, coded as 0-3. Only the two frontal photographs (arms up and down) will be used, and their score will be averaged to produce the outcome measure. **Analysis metric:** The change from baseline at 3 years of follow-up will be used. Negative values will be transformed to '1' to signal an inferiority event, positive or values of zero will be transformed to '0' to signal non-inferiority. **Method of aggregation:** The results will be reported as a contingency table reporting both frequencies and proportions of non-inferior and inferior results in each treatment arm. **Time point(s):** A baseline assessment is performed during the screening visit. The primary endpoint is assessed at 3 year of follow-up after the last study treatment (LST).

	Rationale: This study was designed as a non-inferiority trial. The BCCT.core software offers an objective assessment of aesthetic outcome. By dichotomising the results, they become easier to interpret and compare to the existing literature, however this implies a trade-off in power/sample size.
Cosmetic outcome (Expert panel; AIS-TAS & AIS-Symmetry)	**Operationalisation (measurement variable):** The AIS is a tool for scoring breast cosmesis based on standardized photos. It comprises of 5 items, each comprising of a 5 point Likert-scale, coded as 1 to 5. The score is given based on the group of photos taken at a single time point. The scores of all items are summed to produce the TAS, which consequently ranges from 5 to 25. The AIS is used in an expert panel setting, where all scores per item are averaged and then used as the individual value of this measure. More details can be found in Section 8.7, and Appendix 7. **Analysis metric:** AIS-Symmetry and AIS-TAS absolute values will be used for analysis. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. The primary endpoint is assessed at 3 year of follow-up after the last study treatment (LST). **Rationale:** As discussed in the sample size amendment, this outcome was introduced in order to move away from the dichotomous outcome, and introduce a continuous variable in order to improve the power of this study, for the (second) primary outcome. The AIS-tool was selected due to its simplicity and good inter-rater validity in professionals.
Secondary	
Cosmetic non-inferiority (BCCT.core)	**Operationalisation (measurement variable):** Cfr. supra (BCCT.core primary outcome) **Analysis metric:** Cfr. supra (BCCT.core primary outcome) **Method of aggregation:** Cfr. supra (BCCT.core primary outcome) **Time point(s):** A baseline assessment is performed during the screening visit. The secondary endpoint is assessed at 1 year of follow-up after the last study treatment (LST). **Rationale:** Cfr. supra (BCCT.core primary outcome)
Cosmetic outcome (Physician scoring)	**Operationalisation (measurement variable):** The physician scoring scale (Section 8.7, and Appendix 2) will be used to assess cosmetic outcome. The normalised average score of all items will be summed and used as outcome measure. This will be calculated as the score of an item divided by the amount of answering options minus 1 (k-1), averaged over all items. **Analysis metric:** Absolute values will be used for analysis.

	Method of aggregation: The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. It is then assessed again at 1 year, and 3 years of follow-up after the last study treatment (LST). **Rationale:** This scale offers a concise evaluation of important and relevant items in the assessment of post BCS+WBRT treatment.
Cosmetic outcome (Patient scoring; PROM)	**Operationalisation (measurement variable):** The cosmetic evaluation questionnaire by Sneeuw at al. (Section 8.7, and Appendix 1; Section) will be used to assess cosmetic outcome. The normalised average score of all items will be summed and used as outcome measure. This will be calculated as the score of an item divided by the amount of answering options minus 1 (k-1), averaged over all items. **Analysis metric:** Absolute values will be used for analysis. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. It is then assessed again at 1 year, and 3 years of follow-up after the last study treatment (LST). **Rationale:** This scale is a validated tool for cosmetic assessment.
Quality of life (QLQ – C30 v3)	**Operationalisation (measurement variable):** The QoL (QLQ-C30) outcome variable is operationalised through the QLQ-C30 questionnaire, included in Appendix 3 and discussed in Section 8.8. The answers are transformed into 3 separate scores, which can all range from 0 to 100. These scores are: Global health status/QoL; Functional scales; Symptom scales. Where a higher score on the first 2 scales indicate a better health state and QoL, while on the symptom scale a higher score indicates a higher level of disturbance by symptoms. **Analysis metric:** The QLQ-C30 Global health status/QoL; Functional scales; Symptom scales, absolute values will be used for analysis. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. It is then assessed again at 1 year, and 3 years of follow-up after the last study treatment (LST). **Rationale:** The QLQ-C30 questionnaire offers a validated questionnaire assessing QoL.

Quality of life (QLQ – BR23 v1)	**Operationalisation (measurement variable):** The QoL (QLQ-BR23) outcome variable is operationalised through the QLQ-BR23 questionnaire, included in Appendix 4 and discussed in Section 8.8. The answers are transformed into 2 separate scores, which can both range from 0 to 100. These scores are: Functional scales; Symptom scales. Where a higher score on the functional scale indicate a better health state and QoL, while on the symptom scale a higher score indicates a higher level of disturbance by symptoms. **Analysis metric:** The QLQ-BR23 Functional scales; Symptom scales, absolute values will be used for analysis. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** A baseline assessment is performed during the screening visit. It is then assessed again at 1 year, and 3 years of follow-up after the last study treatment (LST). **Rationale:** The QLQ-BR23 questionnaire, as a subset of the QLQC30 offers a validated questionnaire assessing QoL, specifically pertaining to breast cancer patients.
Local recurrence free survival, and overall survival:	**Operationalisation (measurement variable):** The local recurrence free survival outcome variable is operationalized as recurrence free survival, measured as time to event for local recurrence. The overall survival outcome is operationalized as time to death, paired with disease related death 'yes/no'. As discussed in Section 8.10. **Analysis metric:** The time to event data is registered in days from end of treatment. **Method of aggregation:** The mean, median, variance and IQR will be reported. **Time point(s):** Local recurrence free, and overall survival will be reported at 1, and 3 years of follow-up after the last study treatment (LST). **Rationale:** In order to assess oncological safety of the novel treatment, the local recurrence will be assessed as a secondary outcome variable. The main goal is to observe whether there is a difference, in order to make sure that there is no increase, while also assessing any potential improvement.
Fibrosis	**Operationalisation (measurement variable):** During the study all adverse events (AEs) are monitored and collected in the eCRF, based on the 'National Cancer Institute Common Terminology Criteria for Adverse Events' (NCI-CTCAE) v5.0 reporting system. The AE codes and grades will be recorded in the eCRF and used as variables. The NCI-CTCAE v5.0 and (S)AE registration is discussed in Section 8.9, Section 9, and Appendix 6. For this outcome measure 'Fibrosis' will be tabulated.

	Analysis metric: Tabulation of absence vs. presence and severity will be used for analysis. **Method of aggregation:** The proportion of participants experiencing no 'Fibrosis'-AE vs. any 'Fibrosis'-AE will be used. As well as the frequency of all grades, including 0 (absence). **Time point(s):** AEs will be assessed and recorded continuously, with explicit querying during all follow-up visits. These outcome variables will be reported during interim- and final analyses. **Rationale:** Fibrosis is one of the main radiotherapy comorbidities after BCT, with a significant impact on both cosmetic and breast satisfaction outcomes.
Breast pain	**Operationalisation (measurement variable):** During the study all adverse events (AEs) are monitored and collected in the eCRF, based on the 'National Cancer Institute Common Terminology Criteria for Adverse Events' (NCI-CTCAE) v5.0 reporting system. The AE codes and grades will be recorded in the eCRF and used as variables. The NCI-CTCAE v5.0 and (S)AE registration is discussed in Section 8.9, Section 9, and Appendix 6. For this outcome measure 'Breast pain' will be tabulated. **Analysis metric:** Tabulation of absence vs. presence and severity will be used for analysis. **Method of aggregation:** The proportion of participants experiencing no 'Breast pain'-AE vs. any 'Breast pain'-AE will be used. As well as the frequency of all grades, including 0 (absence). **Time point(s):** AEs will be assessed and recorded continuously, with explicit querying during all follow-up visits. These outcome variables will be reported during interim- and final analyses. **Rationale:** Pain in the treated breast is one of the main radiotherapy comorbidities after BCT, with a significant impact on both cosmetic and breast satisfaction outcomes.

3. Overall study design.

3.1 General scheme of study design

The ULTIMO study is a monocentric, prospective, randomised controlled, non-blinded, phase III interventional clinical trial, with a non-inferiority design, in patients with breast cancer undergoing breast conservative treatment (BCT). After giving informed consent and verifying eligibility, data collection starts and patients will be randomized in one of the following treatment arms:

- **Standard treatment arm**: Breast Conserving Surgery (BCS) takes place, followed by the SoC hypofractioned WBRT and SoC normofractioned sequential boost (normSEB) of 10Gy over 5 fractions.
- **Experimental treatment arm**: Breast Conserving Surgery (BCS) takes place, followed by the SoC hypofractioned WBRT and experimental ultrahypofractionated sequential boost (ultSEB) of 6Gy in a single application.

The primary objective of the ULTIMO study is to assess whether the ultSEB RT boost protocol is non-inferior to the current SoC normSEB RT boost protocol. This assessment will be based on the cosmetic outcome of the breasts, while also taking into account local recurrence rates and the frequency and intensity of (S)AEs.

Participation in the study will comprise a screening period, where the screening assessments must be completed before subjects are enrolled and randomized. Eligible, consenting subjects will then undergo treatment according to their assigned treatment group.

This consists of the SoC therapy for both the surgical and radiotherapeutic WBRT treatments, as appropriate for their specific situation, with a difference in the Boost RT protocol according to the assigned treatment group. Chemotherapeutic or other systemic treatments, either in the neo-adjuvant/preoperative or adjuvant/postoperative setting will be left to the discretion of the treating medical team, there will be no manipulation of this treatment in this clinical trial. Therefore systemic therapies will not be considered study related treatments, however the treatment details will be recorded in the eCRF for post hoc analysis.

After the treatment period is finalized, safety and efficacy are assessed during a follow-up period of 3 years. Follow-up visits will occur at 1 year, and 3 years after treatment finalisation, also known as 'last study treatment' (LST). During this follow-up period, the AEs/SAEs will be registered using the CTCAE V5 (Section 9; Appendix 6). The cosmesis will be evaluated using the self-reported questionnaire by Sneeuw et al., 1992 (5), a physician reported questionnaire after clinical examination, as well as through photographs evaluated by the BCCT.core software package, and a panel of blinded experts (Section 8.7; Appendix 7). The patients quality of life will be assessed using two self-reported questionnaires, the EORTC QLQ-C30 and QLQ-BR23 (Section 8.8; Appendix 3 and Appendix 4).

The screening, treatment and follow-up schedule is shown in Table 1 (SoE).

A schematic presentation of the study design is shown in Figure 1

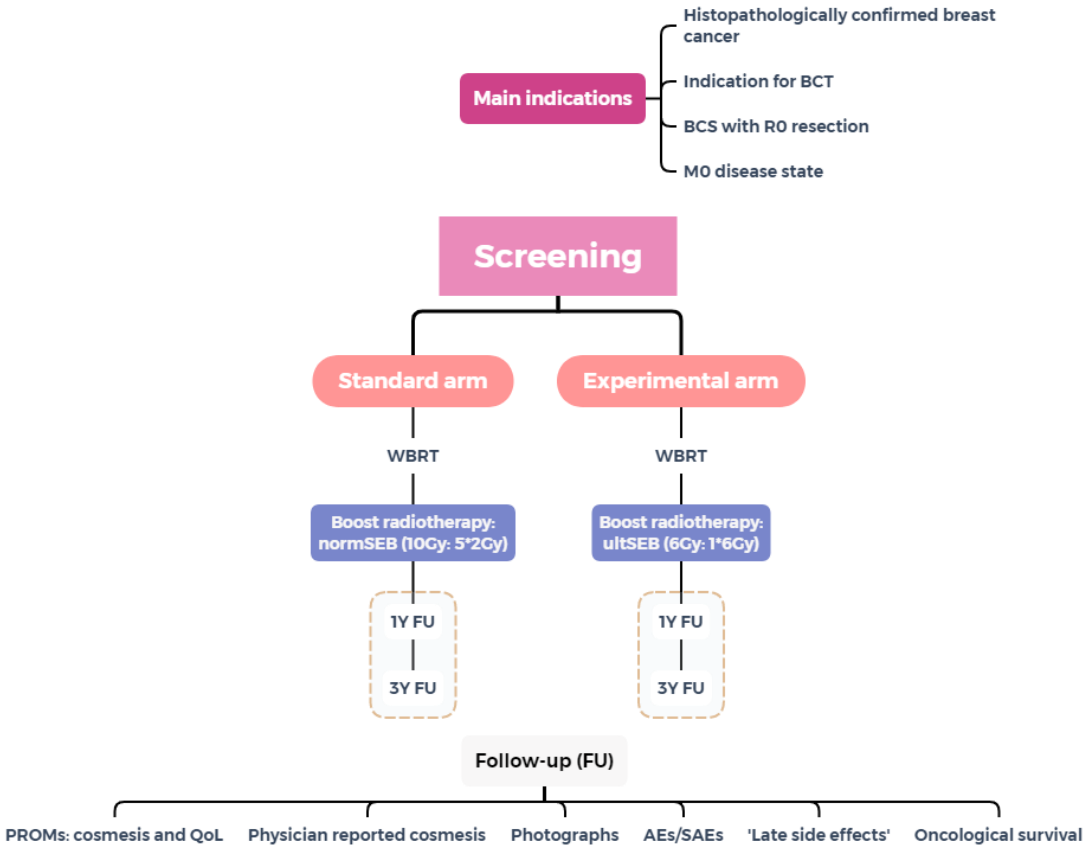

Figure 1. Study design

3.2 Study duration, enrolment and number of sites

The start of the study is defined as the first visit for the first participant providing informed consent. Similarly, the end of study is defined as the last study assessment for the last subject on study or if the sponsor terminates the study, whichever comes first. Primary study completion is defined as the final date on which data for the primary endpoint are expected to be collected. The study duration, with estimated enrolment period of 6-8 months, is expected to be around 4 years.

3.3.1 Total number of study sites/total number of subjects projected

This is a monocentric study. In total, n=132 patients will be included.

4. Eligibility criteria

All women with breast cancer, treated with BCS, referred for postoperative WBRT with an indication for a boost to the lumpectomy cavity according to institute guidelines will be eligible.

4.1 Inclusion criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study:

- Breast cancer patients referred for WBRT + boost to the lumpectomy cavity
- Patients ≥ 18y
- Karnofsky Performance Score > 70%, or ECOG <2
- Life expectancy of more than 5 years
- Invasive tumour free resection margins, defined as R0 resection on pathology report
- Written informed consent

4.2 Exclusion criteria

- Previous contralateral breast cancer
- Prior breast surgery
- Previous RT of the same breast or thorax.
- Metastatic disease (M1)

5. Treatment

5.1 Description

5.1.1 Planning CT scan

A planning CT scan will be performed after surgery in treatment position (supine). CT slices must have a maximum thickness of 3 mm, taken from the bottom of the mandible to several centimetres below the infra-mammary line, including both lungs entirely. Respiratory gating in deep inspiration breath hold will be considered in left-sided breast tumours.

5.1.2. Fractionation and overall treatment time

Using the linear-quadratic model with an α/β value of 3, we estimate 6 Gy in 1 fraction to the tumour bed to be equivalent to the normSEB which is traditionally 10 Gy in 5 fractions. We do not expect increased early toxicity rates since biological equivalent dose remains the same as with normofractionation (1). For late effects the ultSEB might lead to 2 Gy more total dose given.

Patients are randomised to control arm or experimental arm, respectively receiving 5 or 1 daily fractions of 2 or 6Gy each, to the lumpectomy cavity / tumour bed. Preferably, the overall treatment time is 1 week of irradiation in the normSEB group.

The boost dose is given sequential without any gaps after WBRT.

Control arm = WBRT + 10 Gy in 5 fr (normSEB)

Experimental arm = WBRT + 6 Gy in 1 fr (ultSEB)

5.1.3. Target volume

For the WBRT the target area encompasses the complete glandular breast tissue and – if indicated – the lymph node regions according to current ESTRO guidelines.

Organs at risk are contoured, comprising both breasts, both lungs, the heart, the thoracic wall (ribs and musculature), and the skin.

Tumour bed

GTV_boost = In the postoperative setting, there is no true GTV. The tumour bed should be reconstructed (if present and if representative, including the seroma cavity) using adequately placed surgical clips and preoperative imaging (mammogram, MRI, PET/CT) and physical examination if possible. This volume should be as small as reasonable possible.

CTV_boost = The CTV is the rim of breast tissue 1.5 cm around the defined GTV considered at risk for microscopic tumour foci. The CTV margin of 1.5 cm is deducted with the tumour-free margins in all 6 directions as reported in the pathology report. The CTV is adjusted for the thoracic wall and the skin (first 5 mm deep to the external skin contour is excluded).

PTV_boost = An additional margin of 5 mm is added to take into account set-up errors since CBCT is used.

5.1.4 Radiotherapy planning

The tumour bed boost can be delivered by electron or photon beams. When using photon beams this should consist of at least either three or more external photon beams using 6 to 12 MV photons. When using electrons a dose of 6 or 9 MeV is allowed. Bolus is not allowed.

At least 95% of the PTV will be receiving ≥ 90% of the prescribed dose. The dose within the PTV must be within 95% to 101% of the prescribed dose. The 65% isodose should be as conformal as possible around the PTV.

For electrons the 80% isodose should encompass 95% of the PTV.

5.2 Treatment compliance and adherence

In both the control and experimental group daily setup verification is required using electronic portal imaging of the treatment beam. In the experimental group a CBCT is mandatory, in the control group a CBCT at start is mandatory whereafter daily setup verification can be continued with kV of MV imaging.

The electron boost set up is verified daily by visual matching to marks on the skin and checks on the gantry and collimator angles required for matching.

5.3 Concomitant care

Patients will all have completed any type of adjuvant chemotherapy prior to study enrolment.

Adjuvant endocrine therapy, if indicated, will be initiated after completion of the radiation therapy.

If applicable concomitant anti-HER2 therapy is allowed in both study arms, with the exception of ADC therapy in case of non-pCR in HER2 amplified disease.

6. Allocation to treatment groups and blinding

6.1 Assignment of participant number

Each patient is identified in the study by a subject number (subject-id) that is assigned when the patient is first enrolled for screening and is retained as the primary identifier for the patient throughout the entire participation in the trial. The subject-id consists of a sequential patient number, so that each subject is numbered uniquely across the entire database. All subject-IDs will be pseudonymized and secured in a database only accessible by the main researcher.

6.2 Patient randomization

Treatment allocation will be 1:1 and will use random permuted blocks.

7. Study procedures

After treatment clinical follow up should follow local guidelines. For the purpose of the study, assessment of safety (late toxicities) will be incorporated into the follow up visits. Photographs will be taken at baseline and at 1 and 3 years post randomisation. Quality of Life booklets will be completed by patients at baseline, and at 1 and 3 years post randomisation.

The timings and study related procedures are described in the SoE, Table1.

7.2 Screening and randomisation

During the radiotherapy intake consultation, patients will be screened for eligibility (i.e. fulfilling inclusion/exclusion requirements) based on medical record, anamnestic and clinical information from the treating physician. If the patient is found to be eligible, and agrees to participate in the study, the ICF will be explained, the patient will have adequate time and opportunity to ask questions and discuss participation with their partner, family and/or friends. The decision to participate should be free of coercion and will not impact how the patient is treated.

After the decision to include the patient, they receive a pseudonym under which their data will be recorded in the eCRF. During the screening visit, demographic data, health data, and baseline assessments will be recorded, as described in Section 8.

After concluding the baseline assessments, participants will be randomised by the site staff using an electronic randomisation tool (Dyco Capture, DigiDyco).

7.3 Study treatment

Radiotherapy Treatment characteristics will be captured in the eCRF after the last radiotherapy session (see Section 8.1).

7.4 Follow-up visits

During the follow-up visits, at 1 and 3 years of follow-up after LST, photographs of both breasts (digital colour photographs) will be made, as specified in Appendix 5. Both patient and specialist will also complete a cosmesis questionnaire (see Appendix 1 and Appendix 2). Quality of Life will be scored using the EORTC QLQ C30 and BR23 (see Appendix 3 and Appendix 4)

7.5 Subject completion/withdrawal

7.5.1 Subject completion

A subject will be considered as having completed the study if he or she has completed the last follow-up visit (3Y after LST), chooses to withdraw their informed consent and thus to no longer participate in the study, or if they are deceased before the end of the study.

7.5.2 Discontinuation of treatment

Subjects may discontinue the proposed treatment in the following situations:

1. Subject decision. The subject is at any time free to discontinue treatment, without prejudice to further treatment.
2. Severe non-compliance with the study protocol.
3. Disease progression or death.
4. Toxicity experienced as assessed by the investigator.

7.5.3 Criteria for withdrawal from the study

Reasons for withdrawal from the study:

1. Voluntary withdrawal by the subject who is at any time free to discontinue his participation in the study, without prejudice to further treatment.
2. Incorrectly enrolled subjects, i.e. the subject does not meet the required inclusion/exclusion criteria for the study.
3. Subject lost to follow-up.
4. Risk to subjects as judged by the investigator.
5. Severe non-compliance with the protocol.
6. Death.

If a subject is lost to follow-up, every reasonable effort must be made by the study-site personnel to contact the subject and determine the reason for discontinuation/withdrawal. The measures taken to follow-up must be documented.

7.5.4 Subject's withdrawal of informed consent

Subjects are free to withdraw from the study at any time, without prejudice to further treatment. If a participant withdraws from the study, then his/her patient specific number cannot be reused.

If a subject withdraws consent, they will be specifically asked if they are withdrawing consent to:

1. Further participation in the study including any further follow-up.
2. The use of their study generated data.

Regardless of the reason for termination, all data available for the subject at the time of discontinuation of follow-up must be recorded in the electronic case report form (eCRF). All reasons for discontinuation of treatment must be documented.

7.5.5 Study termination

The sponsor reserves the right to terminate any portion of the study at any time. Possible reasons for termination include:

1. Safety reasons
2. New scientific knowledge becomes known that makes the objectives of the study no longer feasible/valid.
3. Unsatisfactory enrolment of participants.
4. Reaching the 3 year follow up time.

In terminating the study, the sponsor will ensure that adequate consideration is given to the protection of the subjects' interests.

7.6 Early termination study visit

NA

8. Study evaluations and measurements

In this section all the data collected from the study participants are discussed. This data is recorded in the eCRF. Initially the eCRF was built in an Excel spreadsheet, from the second amendment onwards, the eCRF was built and recorded in Castor EDC. This decision was made due to the fact that data analysis from the Excel eCRF required multiple data transfer steps with a higher risk of data transfer errors.

The process of transferring the recorded data from the Excel eCRF into the Castor EDC eCRF was performed by trained personnel and data monitoring was performed on all transferred data, as recorded in the new Castor EDC eCRF, in order to avoid errors in data transfer.

8.1 Demographic data

ICF procedure:

- Date of ICF signing
- Protocol version in place at time of ICF signing

Demographics:

- Date of screening visit
- Year of birth
- Age of patient at the moment of study visit
- Biological sex
- Preferred language
- Ethnicity

8.2 Health data

- Menopausal status
- Confirmation of breast cancer diagnosis
- Confirmation of indication for BCS/BCT
- Confirmation of indication for WBRT+Boost
- Karnofsky or ECOG performance status
- Date of performance status assessment
- Life expectancy >5Y Y/N
- Resection margin tumour
- History of breast cancer
- History of radiotherapy to the breast or thorax
- TNM: Metastasis
- Registration of concomitant medications (farmaca, route of administration, dosage, start/stop date)

8.3 RT parameters

The following RT parameters are collected:

- PTV Boost, volume (in cc, no decimals)
- PTV Whole breast, volume (in cc, no decimals)
- PTV Boost V95% (in %, no decimals)
- PTV Boost D95% (in Gy, no decimals)
- PTV Boost D50% (in Gy, no decimals)
- PTV Boost D2% (in Gy, no decimals)

- PTV Whole breast Dmax (in Gy, no decimals)
- CTV Boost, volume (in cc, no decimals)
- CTV Boost V95% (in %, no decimals)
- CTV Boost D95% (in Gy, no decimals)
- CTV Boost D50% (in Gy, no decimals)
- CTV Boost D2% (in Gy, no decimals)
- Breast volume receiving V95% (in cc, no decimals)
- Breast volume receiving V65% (in cc, no decimals)
- Technique (Photons/Electrons
- if electrons, energy used
- Bolus used (Yes/No)

8.4 Systemic treatment details

- Setting: preoperative, and/or postoperative
- Type of systemic therapy, and agents used
- Start/stop dates

8.5 Surgery details

- Date of oncological surgery
- Type of BCS or ME
- Type of reconstruction, in case of ME
- Type of axillary procedure, and nodes removed/nodes positive

8.6 Tumour pathology

- Disease stage (DCIS, Invasive +/- nodal)
- Histological grade
- Histological type
- Uni/multifocal disease
- Tumour size
- Molecular subtype
- Pathological response category

8.7 Assessment of cosmetic outcome

Digital colour photographs will be taken during the screening visit (after BCS, before RT), and 1 year and 3 years after RT. Timing of assessments is based on experience from the START trial, with the aim to maximise the information collected whilst minimising the assessment burden.

Two frontal views of the chest will be taken, one with hands on the hips and the other with hands raised as far as possible above the head, and one profile view (taken from the treated side) with the arms lifted upwards as described in more detail in Appendix 5. All photographs will exclude the patient's head/face. All photographs will be taken and retained by the CTO.

The cosmetic changes will be scored quantitatively by the BCCT.core software program (http://medicalresearch.inescporto.pt/breastresearch/index.php/BCCT.core). Change in breast/reconstructed breast/chest wall appearance and distortion compared with the post-surgical baseline will each be scored on a three-point graded scale. Breast size and surgical deficit will each be assessed on a three-point graded scale from the baseline photographs. Reliability and repeatability of the assessments will be verified (Appendix 5).

The BCCT.core assessment will produce the following variables for each photograph:

- Overall cosmetic score (using BCCT.core)

Furthermore, both patient (Sneeuw et al.) and specialist (Physician scoring scale) will complete validated cosmesis questionnaires during the screening visit, at year one, and three years after treatment (Appendix 1 and Appendix 2, respectively). Each scale will have a single summarising score. This summarising score will be calculated as the score of each item minus one, divided by the amount of answering options minus 1 (k-1) for that item, averaged over all items of the questionnaire. These transformations result in a score ranging from 0 to 100, with 0 representing the worst possible score, and 100 the best possible score.

Score: (RawScore-1)/(k-1), summed over n-items, divided by n, *100

Breast cosmetic assessment based on the AIS tool will be done centrally, by a panel of independent, blinded experts, as well as by a software tool (see Appendix 7).

8.8 Assessment of quality of life

Quality of Life will be scored by using the EORTC QoL questionnaire C30 and BR23, at the screening visit, at year one, and three years after treatment (Appendix 3 and Appendix 4).

The answers are transformed into 3 and 2 separate scores for the C30 and BR23 respectively, which can all range from 0 to 100. These scores are: Global health status/QoL; Functional scales; Symptom scales. Where a higher score on the first 2 scales indicate a better health state and QoL, while on the symptom scale a higher score indicates a higher level of disturbance by symptoms. In the C30 all these scales are included, while in the BR23 the last two scales are included (functional and symptom).
The scales are calculated according to the documentation accompanying the questionnaires, called the 'scoring manual'.

First the RawScore (RS) is calculated:

$RS=(I_1+I_2+\ldots+I_n)/n$

Next, the scale score (S) is calculated:

Functional scales: S= [1 - ((RS-1)/range)]*100

QoL or Symptom scales: S= [((RS-1)/range)]*100

8.9 Assessment of fibrosis and breast pain

All toxicity will be scored according to CTCAE 5.0. Presence of fibrosis/induration will also be scored from grade 0-4, during each follow-up visit. In addition, fibrosis/induration will be scored for the whole breast and the original tumour bed separately (see Appendix 6).

8.10 Assessment of oncological outcome

Physical examination will be performed at every follow-up visit, assessing the loco-regional situation. A control mammography will be performed every year as is standard of care. All patients will be followed until 3 years after RT. The time of follow-up will be counted starting from the date of Randomisation (Rz).

Oncological survival

1. Time to recurrence: will be defined as starting from the date of randomisation (Rz) until the first sign of local recurrence.
2. Time to progression: will be defined as starting from the date of randomisation (Rz) until the first sign of disease progression.
3. Time to death: will be defined as starting from the date of randomisation (Rz) until the date of death. It will also be recorded if death was linked to the oncological disease or not.

9. Safety management and reporting

9.1 Definitions and criteria

9.1.1 Definition of an adverse event (AE)

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject exposed to a clinical study intervention and which does not necessarily have a causal relationship with this intervention. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with a study intervention, whether or not related to the intervention (adapted definition per International Conference on Harmonisation (ICH)).

9.1.2 Definition of a serious adverse event (SAE)

Any adverse event that:

- results in death
- is life-threatening
- requires in-patient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapability
- is a congenital anomaly or birth defect
- is medically important

9.1.3 Definition of a suspected unexpected serious adverse reaction (SUSAR):

An adverse event that is serious and unexpected (meaning that nature or severity of the AE is not consistent with a recognized side-effect of the intervention) and is judged by either the investigator or the sponsor as having a reasonable suspected causal relationship with the study intervention.

9.1.4 Severity criteria

The NCI-CTCAE (most recent version) should be used to grade the severity of adverse events.

9.1.5 Relationship of an SAE to the intervention

The relationship of each SAE to the study intervention should be characterized using one of the following: related or unrelated.

9.2 Reporting procedures

9.2.1 Period of observation

Period	**AEs (non-serious)**	**SAEs**
From: Day of 1 of intervention (RT) To: 3 years after RT	Only if related to trial participation	Only if related to trial participation

9.2.2 Investigator reporting of an (S)AE to the sponsor

Only AEs related to trial participation must be recorded using medical terminology in the source document and the eCRF. Investigators must record in the eCRF their opinion concerning the relationship of the adverse event to the study intervention.

The investigator shall report all serious adverse events that are related to trial participation immediately (<24h), after first knowledge, to the sponsor. Information regarding SAEs will be transmitted to the sponsor using the SAE form, which must be completed and signed by a physician from the study site and transmitted to the sponsor (safetycto@gza.be) within 24 hours.

All SAEs that have not resolved by the end of the study or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- the event resolves
- the event stabilizes
- the event returns to baseline, if a baseline value/status is available
- the event can be attributed to agents other than the study intervention or to factors unrelated to study conduct
- it becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

The immediate and follow-up SAE reports shall identify subjects by patient specific study numbers.

9.2.3 Notification of an SAE to the Ethical Committee

The sponsor shall ensure that all relevant information about suspected unexpected serious adverse reactions that are fatal or life-threatening is recorded and reported as soon as possible to the competent ethics committee, and in any case no later than seven days after knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional eight days.

All other suspected unexpected serious adverse reactions shall be reported to the ethics committee concerned as soon as possible but within a maximum of fifteen days of first knowledge by the sponsor. The sponsor shall also inform the other investigators.

Once a year throughout the experiment, the sponsor shall provide the ethics committee with an annual safety report, listing all suspected serious adverse reactions which have occurred over this period and a report of the subjects' safety (development safety update report). Regarding those adverse events and serious adverse reactions the Principal Investigator will take all reasonable measures, in consultation with Sponsor, to protect subjects at risk following the occurrence of such events.

10. Statistical considerations

The main objective of the current study is to investigate whether an ultSEB results in equal side effects and cosmetic outcome, as compared to a normSEB, without compromising local control. The primary endpoint will be the cosmetic result at 3 years after randomization as assessed by the BCCT.core software program (http://medicalresearch.inescporto.pt/breastresearch/). This software enables quantification of seven features, all associated with fibrosis/induration of the breasts which will then be classified in poor and good cosmetic results (6). From the literature it is known that postoperative radiotherapy is associated with approximately 20% poor cosmetic results (7–11). After expert discussions, it was agreed that an increase of worse outcome of maximum 25% was acceptable.

10.1 Justification of sample size

This is a non-inferiority design with a 1:1 balanced randomisation between the control and experimental groups. The sample size was calculated using a dichotomous (worse vs. equal or improved cosmetic outcome) non-inferiority sample size calculation. The parameters used in this calculation are: Power=80%, 1-sided alpha=5%, control group proportion (worse outcome) =20%, treatment group proportion (worse outcome) =24%, Delta =10%. This resulted in a sample size of n=108. In order to compensate for a 20% lost to follow-up, the sample size was expanded to n=132. The total accrual time is expected to be 1 year.

Amendment to the original sample size calculation:

Due to an inadvertent mistake, the above mentioned sample size does not match the intended calculation. When this calculation was performed again, with power=80%, cosmetic outcome) non-inferiority sample size calculation. The parameters used in this calculation are: Power=80%, 1-sided alpha=5%, control group proportion (non-inferior outcome) =80%, treatment group proportion (non-inferior outcome) =80%, Delta =5%. This resulted in a sample size of n=1583. When the Delta was changed to 10%, the sample size was n=396. Based on post hoc power analysis for these corrected parameters (supra), the power for n=132 and delta=5% is 17.70%, and for delta =10% this is 41.73%.

In light of these findings the AIS-TAS variable was added as a second-primary endpoint, which is a continuous variable, to replace the initial dichotomous primary endpoint.
In order to evaluate the impact of using this second-primary outcome, a new sample size calculation was performed based on this variable. After searching the literature, little data is published on the means and standard deviations of the AIS scores. The original publication by Visser et al., 2010 did publish these results. In this publication, the TAS (total Aesthetic score, sum of all individual items) is not mentioned. Instead the item 'Symmetry' from the postoperative evaluation was selected as a surrogate marker for the TAS score. This decision was based on two reasons: 1) we believe that this captures the essence of aesthetic breast evaluation, which is also mentioned in the same publication as "Panel satisfaction with breast symmetry (rs 0.83), volume (rs 0.82), and shape (rs 0.81) had the strongest effect on satisfaction with the overall aesthetic end result (Table 8)."; 2) this item had the highest standard deviation. The mean was reported to be 3.37 and the standard deviation (SD) was 0.73 (12).

Using the postoperative breast symmetry item from the AIS tool and the 'epi.ssninfc()' function (non-inferiority sample size/power using a continuous variable) in R, the a-priory sample size and post-hoc power were calculated. The arguments were defined as: treatment mean = 3.37, control mean = 3.37, SD = 0.73; Delta = 0.337(10%); n= NA; power = 0.8; nfractional = FALSE, alpha = 0.05. This resulted in a sample size of n=118. When the sample size argument was set to n=132 and power to 'NA', the post hoc power was calculated to be 84,31%.

Next a power analysis was performed for different possible situations, namely treatment mean = 100-90%[*] (2% increment); Delta = 10-15% (1% increment); and n= 132, 120, 110, 100, and 90.
[*](Treatment mean % = Treatment mean/Control mean)

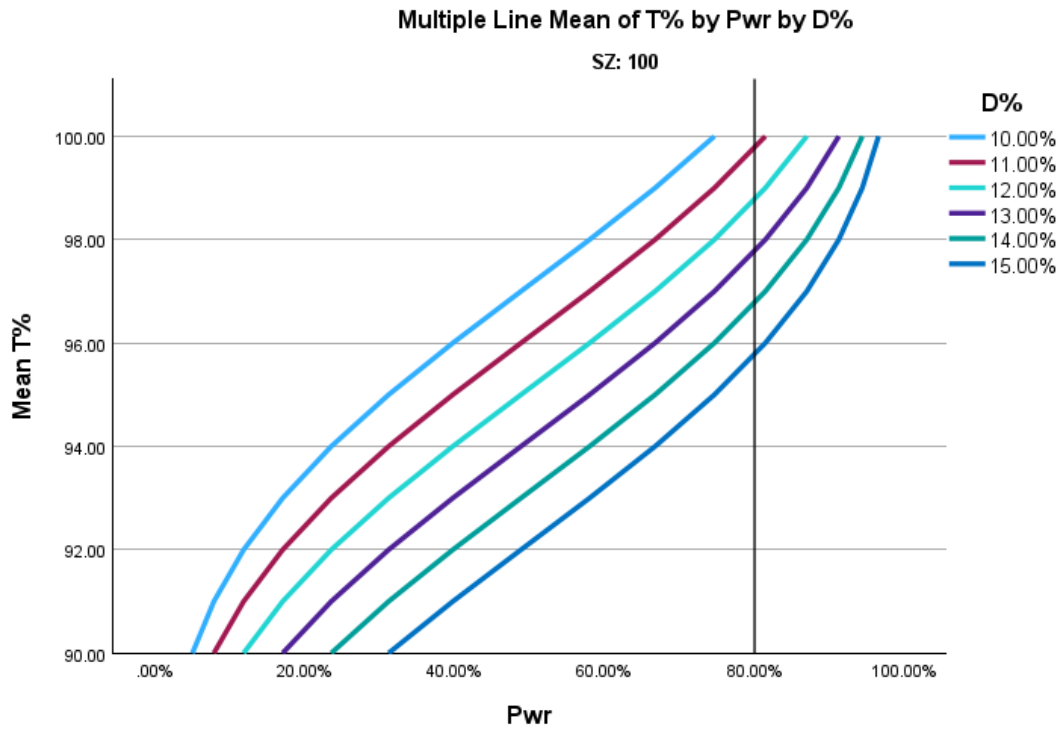

Figure 2: Power analysis

Upon this analysis, and after expert discussion it was decided to set the Delta to 15%. This decision was based on the power analysis showing that for n=110 (which is close to the intended n=108 before dropout margins) a true difference of -4% in treatment mean vs. control mean would still result in sufficient power (>80%), when the delta is defined at 15%. While on the other hand this delta represent an acceptable difference in real life results.

To conclude:

- *The new power analysis based on the newly introduced second primary outcome, starting from the number of participants included in this study states that for n=110, treatment mean = 96%, and Delta = 15%, the calculated power is 84.57%, or 97.65% when both means are assumed to be equal.*
- *Through this adaptation in the (second) primary outcome, switching from a dichotomous to a continuous variable, the study is not underpowered (>80%) based on the collected sample size and post hoc estimated power.*

10.2 Protocol deviations

All protocol deviations will be assessed and documented on a case-by-case basis before database lock. Important protocol deviations related to study inclusion or exclusion criteria, conduct of the study, non-compliance, participant management, or participant assessment should be described. Protocol deviations will be listed, and significant protocol deviations will be reported to the Ethics Committees (EC).

10.3 Statistical methods for analysing primary and secondary outcomes

This sections provides a short and concise overview of the main statistical analyses to be used in evaluating the study endpoints. A full description is provided in the statistical analysis plan (SAP).

Primary outcome – Proportion worse vs. same/improved cosmesis

The change from baseline vs. 3 year follow-up of the BCCT.core overall score (averaged between all three photos) will be recorded as 'inferior' if the value is negative or 'non-inferior' if the value is 0 or positive. The proportion of each category will be calculated and used in the non-inferiority testing.

The Farrington-Manning test for rate difference will be used in 'R'

Primary outcome – AIS-TAS and AIS-Symmetry score

The AIS-TAS and AIS-symmetry scores will be assessed based on the photographs taken during the 3 years follow-up visit (3YFU). The absolute values will be analysed using a t-test based non-inferiority analysis for continuous variables.

Secondary outcomes

All secondary outcomes will be assessed using the Mann-Whitney U test. This approach avoids assumption testing and the pitfalls of the multiple testing problem and corrections.

11. Study management

11.1 Regulatory and ethical considerations

11.1.1 Regulations and guidelines

The investigator is responsible for ensuring that the study is performed in compliance with this protocol, the principles of the Declaration of Helsinki 2024, the current IHC guidelines on Good Clinical Practice (GCP) E6 (R2), and all of the applicable regulatory requirements.

11.1.2 Independent Ethics Committees (IEC)

The clinical trial authorization granted by a favourable opinion from the relevant IEC(s) will be obtained before the start of the study.

At least once a year, the IEC will be asked to review this study. The IEC will be notified about the EoS and a report summarizing the study results will be sent to the IEC within 1 year after the EoS. If the study is terminated early, the IEC will be notified within 15 days.

Favourable opinion is required for the study protocol, protocol amendments, ICFs, participant information sheets, and advertising materials if any.

11.1.3 Insurance and indemnification

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

11.1.4 Risk assessment

Potential risks of study participation

Radiation-induced dermatitis is a minor type of toxicity, further the single boost might give rise to a worse cosmetic outcome on the long term due to a possible increased fibrosis. However, a previous phase 1 study has demonstrated the single ultSEB to be safe and not resulting in a worse cosmetic outcome.

Potential benefits of study participation

The benefit is the shortening in overall treatment time, patients should be coming 4 times less to the radiotherapy department when treated with a single ultSEB.

Risk-benefit assessment

As we expect with modern radiation techniques, the radiotherapy will be safe and effective, the benefits would outweigh the risks of this treatment.

11.2 Informed consent

For each study participant, informed consent will be obtained in writing before any protocol-related activities commence. As part of this procedure, the investigator or a designated representative must explain orally and in writing, by means of the ICF, the nature, duration, the purpose of the study, the number of visits, the assessments, procedures to undergo, and the action of the treatment in such a manner that the participant is

aware of the potential risks, inconveniences, or adverse effects that may occur. Participants should be informed that they may withdraw from the study at any time without any resulting disadvantage and prejudice to their standard treatment care. They will receive all information that is required by national regulations and current ICH and GCP guidelines.

The participant and the investigator will sign the ICF. A copy will be provided to the participant. The originally signed ICF will remain at the study centre. The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained.

All participants will be insured against injury caused by their participation in the study according to the legal requirements. They will be informed about the insurance and the resulting obligations on their part.

11.3 Subject identification, enrolment and screening logs

The investigator agrees to complete a subject identification and enrolment log to permit easy identification of each subject during and after the study. This document will be reviewed by the sponsor study-site contact for completeness. The subject identification and enrolment log will be treated as confidential and will be filed by the investigator in the study file. To ensure subject confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by subject identification and year of birth.

11.4 Data management

11.4.1 Data collection

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfil the objectives of the study.

11.4.2 (Electronic) care report forms

The investigator should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the centre's study participants. Source data should be attributable, legible, contemporaneous, original, accurate, and complete.

All clinical data up until the period between the 1YFU and 3YFU, was captured via electronic data capture using a Microsoft Excel. Due to potential errors in data export from different Excel sheets, as required for data analysis, it was decided to build the eCRF in Castor EDC before the primary endpoint is analysed. The already collected data will be transferred and after data transfer is complete it will again be monitored, in order to protect from data transfer errors. The investigator's study centre staff will enter and edit the data via a secure network. Electronic CRFs will be used for all participants. The investigator's data will be accessible from the investigator's site throughout the study. The eCRF must be kept current to reflect participant status at each part during the course of the study. The eCRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the participant identification and enrolment log. All changes to data are done by the investigator or designated site personnel through the electronic data capture system.

It is the responsibility of the principal investigator of the study centre to ensure that all participant discontinuations or changes in treatment entered on the participant's eCRF are also made on the participant's medical records. The eCRFs for any participant leaving the study should be completed at the time of the final visit or shortly thereafter.

11.4.3 Confidentiality

The data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of subjects confidential.

11.4.4 Record retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all eCRF and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP E6 (R2) Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents. If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor. If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator/institution must permit access to such reports.

11.5 Study monitoring and quality assurance

The sponsor will implement a system to manage quality throughout the design, conduct, recording, evaluation, reporting and archiving of the study with a focus on study activities essential to ensuring protection of participants and the reliability of study results. The quality management system will use a risk-based approach.

The sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study-site visit log that will be kept at the study-site. The first post-initiation visit will be made as soon as possible after enrolment has begun. At these visits, the monitor will compare the data entered into the eCRF with the source documents (e.g., hospital medical records). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the sponsor and study-site personnel and are accessible for verification by the sponsor study-site contact. If electronic records are maintained at the study-site, the method of verification must be discussed with the study-site personnel.

Direct access to source documents (medical records) must be allowed for the purpose of verifying that the recorded data are consistent with the original source data. Findings from this review will be discussed with the study-site personnel. The sponsor expects that, during monitoring visits, the relevant study-site personnel will be available, the source documents will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct. In addition to on-site monitoring visits, remote contacts can occur. It is expected that during these remote contacts, study-site personnel will be available to provide an update on the progress of the study at the site. The study may be audited by the sponsor or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required participant records. By signing this protocol, the investigator grants permission to personnel from the sponsor, its representatives, and appropriate regulatory authorities, for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in eCRF generation, where clinically appropriate.

11.6 Use of information and publication

Results from the study will be submitted for publication in high ranking journals in the field of radiotherapy, breast cancer and/or oncology.

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Appendices

Appendix 1: Patient's questionnaire, cosmesis

Adapted from Sneeuw et al. (1992a) (5)

The following questions relate to the appearance of the treated breast, and how you experience possible changes in appearance.

1. What is your opinion on the visibility of the scar?
    a) the scar is hardly visible
    b) the scar is somewhat visible
    c) the scar is clearly visible
    d) the scar is very visible
2. What is your opinion on the size of the treated breast compared to the untreated breast?
    a) no difference
    b) a small difference
    c) a moderate difference
    d) a large difference
3. What is your opinion on the shape of the treated breast compared to the untreated breast?
    a) no difference
    b) a small difference
    c) a moderate difference
    d) a large difference
4. What is your opinion on the firmness of the treated breast compared to the untreated breast?
    a) no difference
    b) a small difference
    c) a moderate difference
    d) a large difference
5. What is your opinion on the colour of the skin of the treated breast compared to the untreated breast?
    a) no difference
    b) a small difference
    c) a moderate difference
    d) a large difference
6. What is your opinion on the position of the nipple of the treated breast compared to the untreated breast?
    a) no difference
    b) a small difference
    c) a moderate difference
    d) a large difference
7. How do you feel in general about the appearance of the treated breast compared to the untreated breast?
    a) no difference
    b) a small difference
    c) a moderate difference
    d) a large difference
8. How satisfied/unsatisfied are you with the appearance of the treated breast compared to the untreated breast?
    a) very satisfied
    b) a bit satisfied
    c) not unsatisfied
    d) unsatisfied
    e) very unsatisfied

Appendix 2: Physician's questionnaire, cosmesis

VISIBLE SEQUELS

(Treated breast versus contralateral breast)

Breast size

0 = no difference
1 = a small difference
2 = a moderate difference
3 = a large difference

Nipple position

0 = no difference
1 = a small difference
2 = a moderate difference
3 = a large difference

Shape of the areola and nipple

0 = no difference
1 = a small difference
2 = a moderate difference
3 = a large difference

Skin color

0 = no difference
1 = a small difference
2 = a moderate difference
3 = a large difference

Appearance of the surgical scar

0 = very unobtrusive
1 = visible, but not affecting cosmesis
2 = visible and detracting somewhat from cosmesis;
3 = visible and detracting a great deal from cosmesis
96 = not evaluable

TELANGIECTASIA

Telangiectasia in RT area

0 = not visible
1 = a few < 1 cm^2
2 = clearly visible: 1-4 cm^2
3 = severe: > 4 cm^2
96 = not evaluable

GLOBAL COSMETIC RESULT

Global cosmetic result

0 = excellent
1 = good
2 = fair
3= poor
96 = not evaluable

Do you expect improvement with additional surgery of the opposite breast?

0 = no
1 = only slightly
2 = moderately
3 = a great deal

Appendix 3: EORTC QLQ-C30

ENGLISH

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ☐☐☐☐

Your birthdate (Day, Month, Year): ☐☐ ☐☐ ☐☐☐☐

Today's date (Day, Month, Year): 31 ☐☐ ☐☐ ☐☐☐☐

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Appendix 4: EORTC QLQ-BR23

EORTC QLQ - BR23

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week.

During the past week:	Not at All	A Little	Quite a Bit	Very Much
31. Did you have a dry mouth?	1	2	3	4
32. Did food and drink taste different than usual?	1	2	3	4
33. Were your eyes painful, irritated or watery?	1	2	3	4
34. Have you lost any hair?	1	2	3	4
35. Answer this question only if you had any hair loss: Were you upset by the loss of your hair?	1	2	3	4
36. Did you feel ill or unwell?	1	2	3	4
37. Did you have hot flushes?	1	2	3	4
38. Did you have headaches?	1	2	3	4
39. Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
40. Have you been feeling less feminine as a result of your disease or treatment?	1	2	3	4
41. Did you find it difficult to look at yourself naked?	1	2	3	4
42. Have you been dissatisfied with your body?	1	2	3	4
43. Were you worried about your health in the future?	1	2	3	4

During the past four weeks:	Not at All	A Little	Quite a Bit	Very Much
44. To what extent were you interested in sex?	1	2	3	4
45. To what extent were you sexually active? (with or without intercourse)	1	2	3	4
46. Answer this question only if you have been sexually active: To what extent was sex enjoyable for you?	1	2	3	4

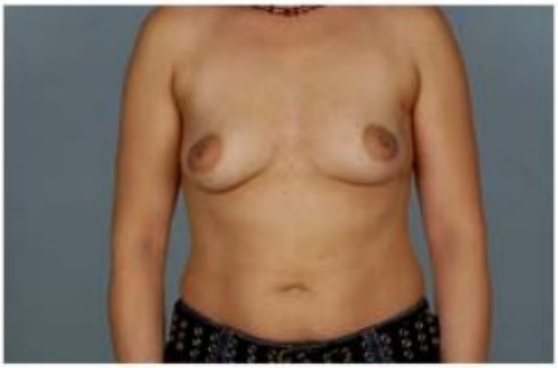
During the past week:	Not at All	A Little	Quite a Bit	Very Much
47. Did you have any pain in your arm or shoulder?	1	2	3	4
48. Did you have a swollen arm or hand?	1	2	3	4
49. Was it difficult to raise your arm or to move it sideways?	1	2	3	4
50. Have you had any pain in the area of your affected breast?	1	2	3	4
51. Was the area of your affected breast swollen?	1	2	3	4
52. Was the area of your affected breast oversensitive?	1	2	3	4
53. Have you had skin problems on or in the area of your affected breast (e.g., itchy, dry, flaky)?	1	2	3	4

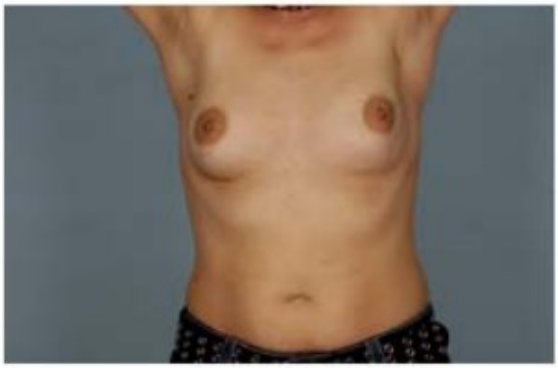
Appendix 5: Photographs

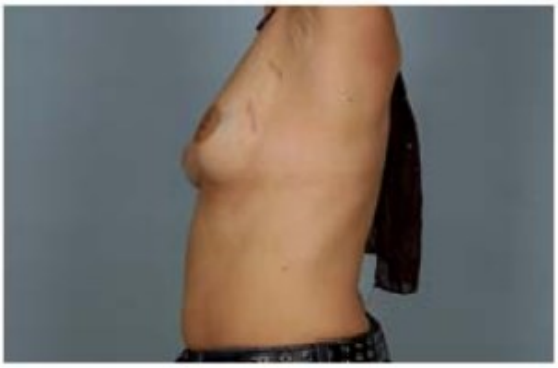
The cosmetic outcome and assessment of therapy sequels will be scored regularly. Therefore digitalized colour photographs have to be taken immediately before local treatment and 1 and 3 years after randomization.

The photographs should be taken with a digital camera (minimum 1,3 megapixels) without flash, in a room with no direct daylight. Photographs must be taken with the patient in upright position, standing in front of a homogenously coloured wall. The photographs should be two frontal views, one with the arms lifted upward, one with the arms along the body, and one profile view (taken from the treated side) with the arms lifted upwards is mandatory, without jewellery. The cosmetic changes will be scored quantitatively (on the -frontal view with the arms down- photographs) by the BCCT.core software programme. http://medicalresearch.inescporto.pt/breastresearch.

This software enables quantification of seven features (pBRA = change in nipple position, pLBC = change in level of lower breast contour, pUNR = change in nipple level, pBCE = change in distance from nipple to inframammary fold, pBCD = change in length of breast contour, pBAD = change in area of the breast, pBOD = change in non-overlapping area between left and right breast), all associated with fibrosis of the breasts (6). With this software programme it is also possible to measure skin colour changes and scar visibility, and to extract an overall cosmetic score. Skin telangiectasia should be scored separately, according to Bentzen et al. (13) i.e. grade 0 = no telangiectasia, grade 1 is < 2 telangiectasia/cm2, grade 2 is 2-4 telangiectasia/cm2 , grade 3 is > 4 telangiectasia/cm2.







Appendix 6: CTCAE V5

The Cancer Therapy Evaluation Program NCI-CTCAE version 5.0 (November 27, 2017) can be viewed online at the following NCI website:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

Appendix 7: The aesthetic Items Scale (AIS)

For the expert panel assessment of the photographs taken at baseline and during follow-up visits, the AIS tool will be used. In this process, the scores from a blinded panel of independent observers (consisting of at least 2, ideally 5 physicians with a background in either plastic surgery or mammary radiotherapy), using the 'The aesthetic Items Scale' will be collected and the average calculated. Inter- and intra-observer agreement of the ratings between the observers will be expressed as intraclass correlation coefficients (ICCs) with corresponding 95% confidence intervals. An ICC of >0.7 will be considered to indicate a good reliability agreement (14).

Twenty randomly selected photographs (not included in the actual study) will be shown to the panel before scoring begins in order to avoid skewness between observations.

In the aesthetic items scale, the breasts are evaluated with respect to volume, shape, symmetry, scars, and nipple areola complex. For each of these items a 5-point Likert scale is used for scoring. This scale ranges from "very dissatisfied," "dissatisfied," "neutral," "satisfied," to "very satisfied." The Total Aesthetic Score (TAS), is derived by summing the score of the 5 items (15).

The TAS will be used for outcome operationalisation.

Item N°	Score:	1	2	3	4	5
1	Volume	Very dissatisfied	Dissatisfied	Neutral	Satisfied	Very satisfied
2	Shape	Very dissatisfied	Dissatisfied	Neutral	Satisfied	Very satisfied
3	Scars	Very dissatisfied	Dissatisfied	Neutral	Satisfied	Very satisfied
4	Nipple (areola complex)	Very dissatisfied	Dissatisfied	Neutral	Satisfied	Very satisfied
5	Symmetry	Very dissatisfied	Dissatisfied	Neutral	Satisfied	Very satisfied

Appendix 8: Karnofsky and ECOG score

Karnofsky Status	Karnofsky Grade	ECOG Grade	ECOG Status
Normal, no complaints.	100	0	Fully active, able to carry on all pre-disease performance without restriction.
Able to carry on normal activities. Minor signs or symptoms of disease.	90	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
Normal activity with effort.	80	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
Care for self. Unable to carry on normal activity or to do active work.	70	2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
Requires occasional assistance, but able to care for most of his needs.	60	2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
Requires considerable assistance and frequent medical care.	50	3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
Disabled. Requires special care and assistance.	40	3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
Severly disabled. Hospitalisation indicated though death nonimminent.	30	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
Very sick. Hospitalisation necessary. Active supportive treatment necessary.	20	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
Moribund	10	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.

This table stems from the 'European Association of Urology Guidelines on Prostate Cancer 2015' by Mottet et al., 2015 (16), which is an adaptation of the original table by Oken et al., 1982 (17)

Appendix 9: Amendment history

All previous versions of amendments will be collected in this appendix, the overview of changes relating to the current version of the protocol will be displayed at the beginning of this document. All previous amendment overviews will be kept in this appendix.

History of the protocol and its amendments	
Protocol version	**Date of IEC application**
Protocol v1.0, original protocol	17-FEB-2022
Protocol v2.0, amendment	20-SEP-2022
Protocol v3.0, amendment	Current review

Protocol v2.0, amendment 1 (20-SEP-2022)

The purpose of this amendment is to increase the number of patients to be included in the study.

Changes to the protocol:

Changes to specific sections of the protocol are shown in the track changes version of the protocol using strike through red font for deletions and red font for insertions.

The changes made to the protocol due to this amendment are incorporated in the following sections:

- Study synopsis: the number of patients was changed into 132.
- Figure 1 Study design: the number of patients was changed into 132.
- Section 3.3 'Study duration, enrolment and number of sites': the number of patients was changed into 132.
- Section 10.1 'Justification size': the number of patients was changed into 132.

Protocol v3.0, amendment 20-MAY-2025

Purpose of this amendment:

1. Amendment to the sample size calculation.
2. Addition of a second primary outcome to improve the expected power after sample size amendment.
3. Improved definition of the statistical methodology.
4. Overhaul of general aspects of the protocol, to improve readability and clarity.
5. Removal of secondary outcome "Health economic impact".

A summarized list of key changes:

Section Number and Name	Description of change and Brief Rationale
Cover page + Title + Statement of compliance + Confidentiality statement + Roles and responsibilities + Funding + Sponsor's Approval + Investigator Agreement	**Description of change:** - Re-arrangement of titles - Added details **Brief rationale:** - These changes are compliant with the SPIRIT

	2013 statement, and improved information/details.
Table of contents + List of tables and figure / attachments / abbreviations + amendments	**Description of change:** - Updated **Brief rationale:** - Updated, cfr. other changes
Synopsis + Schedule of Events	**Description of change:** - Updated - Addition of a SoE **Brief rationale:** - Updated, cfr. other changes - A SoE provides an improved overview of how the study is organised, compliant with SPIRIT 2013
1. Background & Rationale	**Description of change:** - Minor grammatical and layout changes **Brief rationale:** - Improved clarity
2. Study objectives and endpoints	**Description of change:** - Changed to the table regarding study objectives and endpoints in the synopsis section. - Removal of secondary outcome “Health economic impact”. - Minor grammatical and layout changes **Brief rationale:** - More detailed and uniform description of the endpoints and their operationalisation. - This secondary endpoint was not operationalised at the onset of the study, the data not collected and data collection would at this point take too much effort for the potential gain that it offers. - Improved clarity
3. Overall study design.	**Description of change:** - Rewritten this section to include a complete overview of how the study will be conducted - Changed the study setup diagram **Brief rationale:**

	- This was not yet included - The previous diagram contained discrepancies in participant numbers
4. Eligibility criteria	**Description of change:** - Added ECOG, next to the Karnofsky score - Added clarification that tumour free resection margins are defined as an 'R0' resection on the pathology report. - Minor grammatical and layout changes **Brief rationale:** - The ECOG is more widely used. A Karnofsky-ECOG transformation table was added in the Appendices - Improved clarity
5. Treatment	**Description of change:** - Section on how the equivalent dose was calculated, is moved from section to, to section 5 - Minor grammatical and layout changes **Brief rationale:** - Section 5 is the adequate section on this topic. - Improved clarity
7. Study procedures	**Description of change:** - Time and event table was removed, a schedule of events was created and added as a separate heading. - Minor grammatical and layout changes **Brief rationale:** - Original table was too concise, the SoE provides a more detailed, yet clear overview. - Improved clarity
8. Study evaluations and measurements	**Description of change:** - The topics of demographic and health data were added, describing which data was recorded in the eCRF - All data recorded in the eCRF is described under the appropite subsection - Minor grammatical and layout changes **Brief rationale:**

	- Improved data transparency - Improved clarity
10. Statistical considerations	**Description of change:** - Amendment of the sample size calculation - Amendment of statistical analysis methodology - Minor grammatical and layout changes **Brief rationale:** - A mistake was discovered - Appropriate statistical tests were introduced. - Improved clarity
11. Study management	**Description of change:** - DoH v2024 - GCP E6 (R2) - Removal of superfluent paragraph - Details on how the eCRF will be changed from excel to Castor EDC **Brief rationale:** - Improved details - Removal of superfluent details - The excel presented challenges for safe data transfer, required for data analysis.