Danish Breast Cancer Group, DBCG

The DBCG PROTON Trial.
Adjuvant breast proton radiation therapy for early breast cancer patients: The Skagen Trial 2, a clinically controlled randomised phase III trial

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CONTENT

1.0 BACKGROUND

Breast cancer is the most frequent non-skin cancer among women in DK with 4700 patients diagnosed yearly. Around 95% of patients are diagnosed with curable disease. The Danish Breast Cancer Group (DBCG) was established in 1976 to register patient, tumour and therapy related data together with monitoring outcome and developing new treatment strategies in an evidence-based manner (1).

Adjuvant breast cancer radiation therapy (RT) is standard for all patients operated with breast conservation and for patients diagnosed with large tumours (pT3-4) and/or node-positive disease. In Denmark, 3500 breast cancer patients have adjuvant RT yearly, and around 65% of all breast cancer patients treated with RT have whole breast RT without nodal RT, whilst the remaining 35% are treated with loco-regional RT (target is breast / chest wall and regional nodal volumes). RT leads to fewer local and regional recurrences, a decrease in breast cancer death and improves overall survival. Since June 2014, when the DBCG IMN study showed overall survival gain from internal mammary node (IMN) RT, IMN RT has been standard for all high-risk patients (2-4). IMN RT causes a significant increase in dose to the heart and lung, thus heart and lung sparing RT techniques based on deep inspiration breath hold (DIBH), volumetric arc therapy and tomotherapy are increasingly used to lower dose to heart and lung whilst maintaining dose to breast and nodal targets (5), Figure 1. These advanced techniques are used in all DBCG departments routinely. Despite using advanced RT techniques, some patients still receive high RT dose to heart and lung.

The prognosis for most breast cancer patients has fortunately improved considerably over the last decades so many patients stay alive for decades after diagnosis and treatment. Serious life-threatening late effects are radiation-induced heart disease and second cancer, most frequently lung cancer. A dose-response effect between dose to heart and/or lung exists, and for both morbidities, these serious late events become clinically relevant for patients surviving >10 years (6,7). RT may cause cardiac damage to any part of the heart either acutely or as a late effect including but not limited to coronary artery disease, acute myocardial infarction, acute heart failure due to cardiomyopathy and calcification of valves. In irradiated breast cancer patients, coronary artery disease appears to be the most frequently seen late morbidity (8-11), Figure 2. Taken together, there is a delicate balance between gain and risk from adjuvant breast cancer RT, and in most high-risk patients this leads to compromises on dose coverage of target volumes to decrease dose to critical organs at risk.

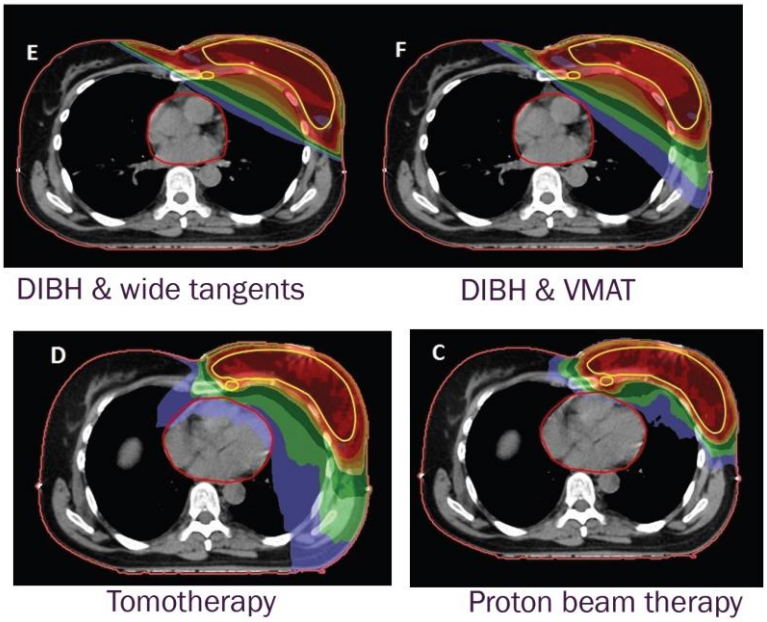
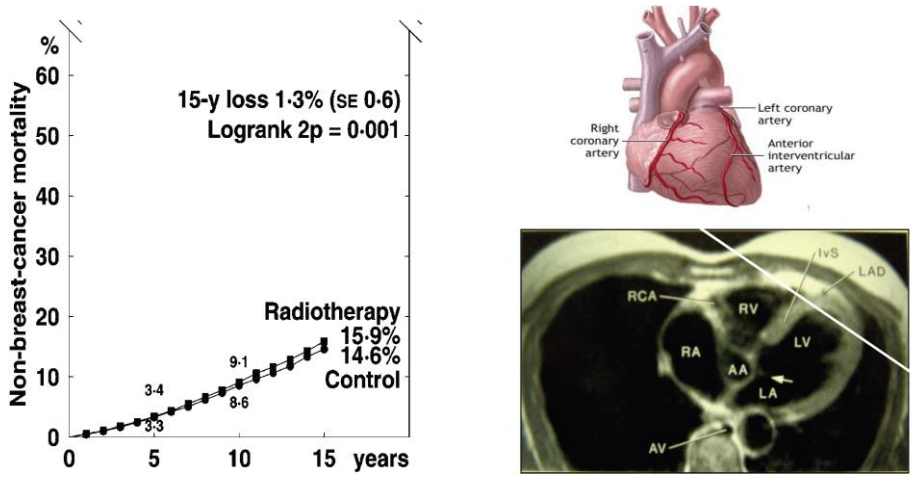


Figure 1 illustrates dose distribution using different RT techniques



Early Breast Cancer Trialists' Collaborative Group, Lancet, 2005

Figure 2, illustration of cardiac risks using left-sided breast cancer RT

Proton therapy and early breast cancer

Proton therapy (PT) has not been widely used nor investigated for adjuvant breast cancer RT, because there are only few proton centres across the world. However, due to the properties of PT it is possible to achieve optimal dose coverage of relevant targets and at the same time ensure low dose to organs at risk compared with photon RT. In an energy-dependent manner, PT will deposit the majority of its dose in tissue depths defined by the Bragg peak (Figure 3). In practice, this translates into i) the ability to deliver the peak energy to target volumes of irregular 3-dimensional shape using pencil-beam scanning technology, ii) a sharp dose fall-off following deposition of energy in the target and iii) reduction of the integral dose to the patient. Within millimeters, the exit dose drops off from 90% to 10%, resulting in the virtual absence of an exit dose. The effectiveness, safety and feasibility of PT has been reported in few small cohort studies with limited follow up, and there is a lack of clinically controlled randomised trials documenting benefit from PT, evaluated either as higher tumour control and/or as fewer morbidities.

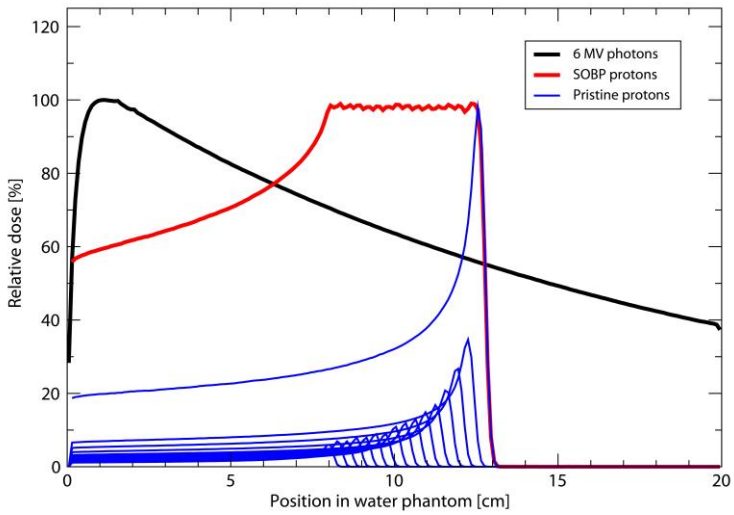


Figure 3 illustrated the dose-depths relationship of protons versus photons

Stick et al. estimated the gain from better target coverage and less dose to organs at risk using PT in unselected high-risk breast cancer patients treated according to DBCG guidelines (12). In 41 consecutive left-sided high-risk patients a joint estimate of risk of recurrence from inadequate dose to nodal targets and the risk from cardiac toxicity from RT exposure to the heart in photon plans compared with PT plans was evaluated. The authors found that PT plans were superior in all patients compared with photon plans (Figure 4), and they estimated that the excess absolute risk of major coronary event or cardiac death was median 1.0% (0.2-2.9%) / 0.5% (0.03-1.0%) with / without cardiac risk factors, but even lower with PT 0.13% (0.02-0.5%) / 0.06% (0.004-0.3%), respectively. The median excess absolute risk of breast cancer recurrence after 10 years was 0.10% (range, 0-0.9%) with photons and 0.02% (range, 0-0.07%) with protons. The authors concluded that modern photon therapy carries a risk of RT-induced heart disease, but PT can reduce this risk. Undoubtedly, the gains and risks from RT will become higher if patients are selected for PT. The DBCG strategy is therefore to select the high-risk patients with highest dose to heart/lung (see later).

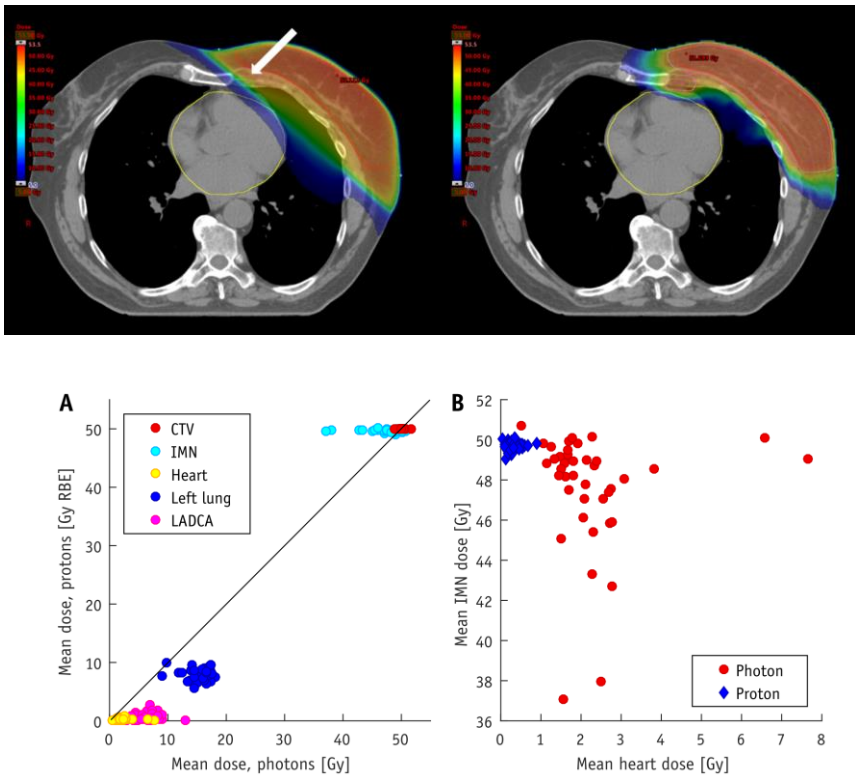


Figure 4, Illustrations from Stick et al (12)

There are three main goals using PT in early breast cancer, i) PT can provide maximum sparing of heart and coronary arteries, thus reduce the risk of RT-induced cardiac disease and death, ii) PT can reduce the integral volume of normal tissue exposure to RT and thereby reduce risk of second cancer (second lung cancer or in young women RT-induced contralateral breast cancer), iii) PT ensures optimal dose coverage of target volumes including the IMN, improving local, regional and distant control and improving overall survival.

Few studies have focused on PT as loco-regional RT of early breast cancer (13-17), Table 1. These cohort studies unanimously reported good tolerance regarding acute toxicity with no increased skin and cosmetic toxicities using standard normo-fractionated loco-regional PT. They also confirmed in clinical use that the mean heart dose indeed was very low even in left-sided IMN treated patients, and no cases with pneumonitis have been reported so far. Patients with implant-based immediate reconstruction were treated with protons without unexpected acute morbidities, except one study reporting an increased risk of reconstruction failure using moderately hypo-fractionated proton therapy (RR 4.99, 95% CI 1.24-20.05) (13). In general,

the studies are small, follow up extremely short, and most patients had PT based on concern for heart morbidity although no strict cut-off values for offering the patient PT were provided.

Author	Reason for PT	Selection	N	Period	Chemo therapy	ALND	Follow up, median	Left/ Right	Immedi. Recon.
Smith et al (13) Mayo Clinic	Concern over heart	Immediate breast reconstruction and need for PMRT	51	2015-2017					100%
Luo et al 2018 (14) New York	Concern over cardiac or lung	Early BC or chest wall recurrence with need for PMRT	42	2013-2015	43% neoadj, no information on adj	79%	35 months (1-55)	86% / 14%	62% (96% implant)
Verma et al (15) Chicago	Concern over cardiac or lung	LABC 29% BCS 71% mastectomy	91	2011-2016	51% neoadj 46% adj	51%	15.5 months	62% / 36% / 2% bilat	33%
Bradley et al (16) Florida	Concern over heart	Require RNI Stage II-III 39% lumpectomy 61% mastectomy	18	2012-2014	94% had chemo (56% were LABC and had neoadj, 33% adj, 6% concurrent and adj)	Not reported	20 months (2-31)	50% / 50%	22%
MacDonald et al (17) Boston	V20 heart ≥5% and/or LAD≥20 Gy with photons. Also immediate recon if suboptimal plan (no expanders allowed)	Require PMRT, 92% had reg RT also and this included IMN due to LABC	12	Not reported	42% neoadj 58% adj	Not reported	Followed until 8 weeks after end RT	92% / 8%	42% All implants

Table 1

Active trials during 2019 investigating loco-regional PT

Currently, few studies investigating adjuvant PT for early breast cancer are running. In 2013 a multi-institutionally American phase II trial was initiated (*NCT 01758445*) investigating the cardiac-sparing potential of PT for stage II-III breast cancer patients requiring loco-regional RT irrespective of lumpectomy or mastectomy. The primary endpoint is acute and late radiation effects. It plans to accrue 220 patients during 2013-2022. The RADCOMP trial (*NCT 02603341*) is a pragmatic randomized phase III trial testing PT vs photon RT for patients with stage II-III breast cancer (18). Primary endpoint is major coronary event (MCE) reduction by PT (hypothesizing a reduction in the 10-year MCE rate from 6.3 to 3.8% compared to photons). The trial aims for 1278 patients accrued during 2016-2020 (2019: 700 patients accrued). The RTOG 3510 trial (*NCT 03270072*) is a phase III trial from Boston randomizing PT vs photon RT in 100 patients. The primary endpoint is change in echocardiographic global longitudinal strain. Finally, the Mayo Clinic randomises 15 vs 25 fractions PT after mastectomy aiming at 109 patients during 2016-2020, and the primary endpoint is grade 3 late effects (*NCT 02783690*).

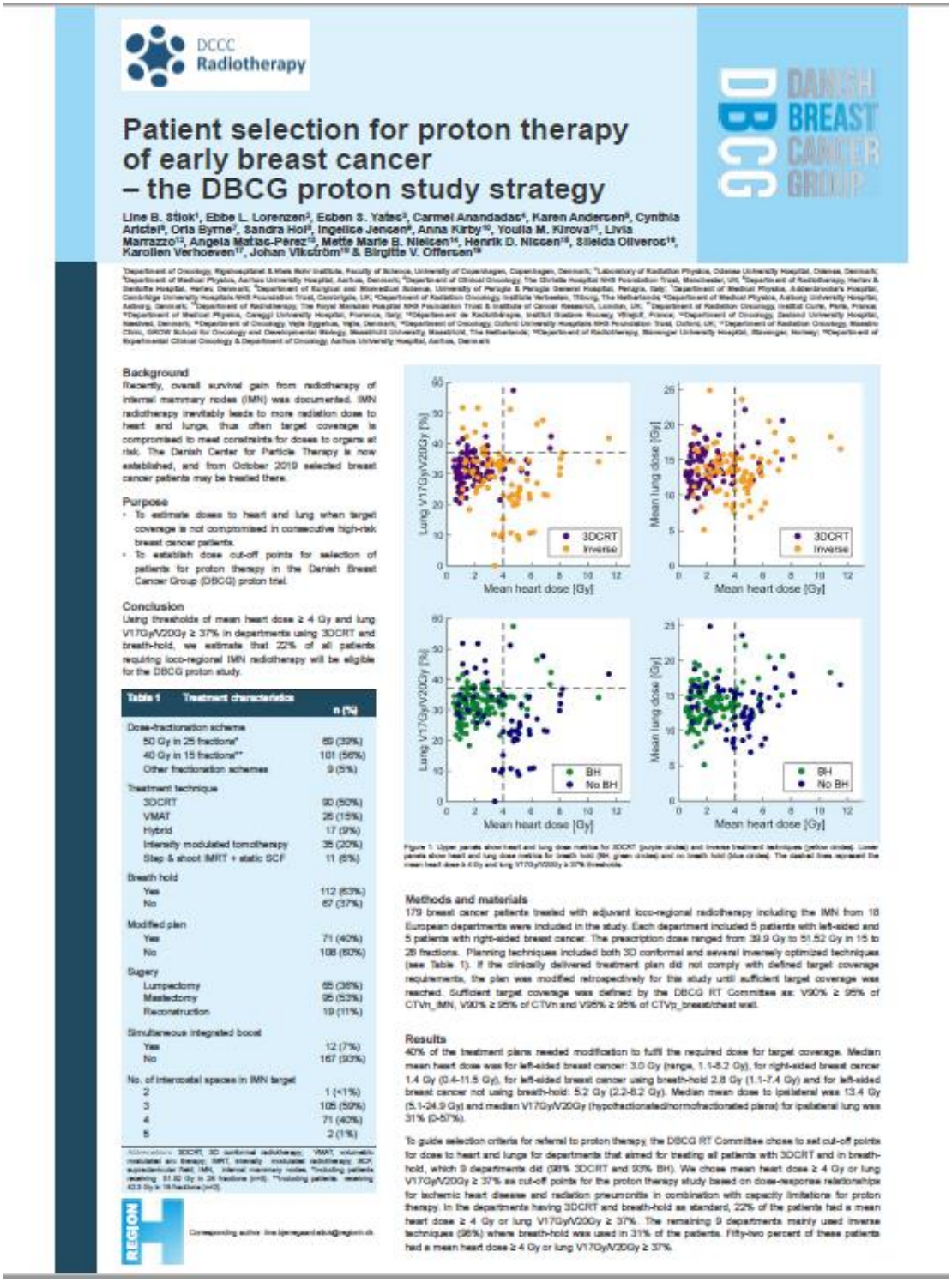
Establishing DBCG selection criteria for PT

Since Jan 2019, selected cancer patients have PT at the Danish Center for Particle Therapy (DCPT). Proton therapy is not standard for early breast cancer, and it will only be possible to provide PT for breast cancer patients at the DCPT inside a trial. The DBCG RT Committee has therefore made a strategy for implementing PT in breast cancer in DK. A retrospective study based on RT plans from 18 European RT centers was performed (Stick et al, ESTRO38 manuscript in preparation), Figure 5, and showed that the present protocol should be a phase III study – The DBCG PROTON Trial – which will include patients where standard RT (defined here as V90% ≥ 95% of CTV_IMN, V90% ≥ 95% of CTVn and V95% ≥ 95% of CTVp_breast/chest wall) causes a mean heart dose (MHD) ≥4 Gy and / or a V20 lung ≥ 37%. These selection criteria identifies estimated 245 patients per year corresponding to 20% of all high-risk breast cancer patients in DK and 7% of all breast cancer patients treated with RT.

In a cohort of 90 patients, 20 high risk breast cancer patients (22%) fulfilled one or both selection criteria: 9 patients (45%) were selected on heart constraint, 8 patients (40%) were selected on lung constraint, and 3 patients (15%) were selected on both constraints.

The selection criteria were also discussed at the Skagen Meeting June 2019, and they are likely to be used also in the planned breast PT trials in UK and F.

The results from the retrospective study were discussed during several online meetings in the DBCG proton group. There was a very high agreement among all DBCG departments using 3D-CRT and breath hold technique in almost every patient. Given a yearly capacity at the DCPT of 200 breast cancer patients, it was decided that selection criteria for proton therapy in the DBCG phase III trial are MHD ≥4 Gy and/or V17/V20lung ≥ 37% measured in a treatment plan according to the criteria listed in the abstract above. These criteria will identify around 7% of all breast cancer patients in Denmark (corresponding to 245 patients). However, not all patients are expected to accept referral for PT, so the actual yearly number is expected to be around 200 patients. The far majority of patients with high heart/lung radiation dose will have an indication for IMN RT, and therefore they are candidates for loco-regional RT. Only very few patients treated with breast only RT will have such high doses to heart/lung, but it may be the case in patients with special anatomy or if the tumour bed is very close to the heart, and such patients are also accepted in the trial.
The median age of breast cancer diagnosis in Denmark is 61 years, and only few patients are <40 years at diagnosis. The risk of radiation induced contralateral breast in patients <40 years is relatively high. It has been demonstrated that if the medial quadrants of the contralateral breast receive >1.0 Gy mean dose, the RR for developing a contralateral breast cancer is 2.5, 95% CI 1.4-4.5 (19). Thus, in this trial special focus on contralateral dose is needed for young patients.



Patient selection for proton therapy of early breast cancer – the DBCG proton study strategy

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Background
Recently, overall survival gain from radiotherapy of internal mammary nodes (IMN) was documented. IMN radiotherapy inevitably leads to more radiation dose to heart and lungs, thus often target coverage is compromised to meet constraints for doses to organs at risk. The Danish Center for Particle Therapy is now established, and from October 2019 selected breast cancer patients may be treated there.

Purpose
- To estimate doses to heart and lung when target coverage is not compromised in consecutive high-risk breast cancer patients.
- To establish dose cut-off points for selection of patients for proton therapy in the Danish Breast Cancer Group (DBCG) proton trial.

Conclusion
Using thresholds of mean heart dose ≥ 4 Gy and lung V17Gy/V20Gy ≥ 37% in departments using 3DCRT and breath-hold, we estimate that 22% of all patients requiring loco-regional IMN radiotherapy will be eligible for the DBCG proton study.

Table 1 Treatment characteristics	n (%)
Dose-fractionation scheme	
50 Gy in 25 fractions*	69 (39%)
40 Gy in 15 fractions**	101 (56%)
Other fractionation schemes	9 (5%)
Treatment technique	
3DCRT	90 (50%)
VMAT	26 (15%)
Hybrid	17 (9%)
Intensity modulated tomotherapy	35 (20%)
Step & shoot IMRT + static SCF	11 (6%)
Breath hold	
Yes	112 (63%)
No	67 (37%)
Modified plan	
Yes	71 (40%)
No	108 (60%)
Surgery	
Lumpectomy	65 (36%)
Mastectomy	95 (53%)
Reconstruction	19 (11%)
Simultaneous integrated boost	
Yes	12 (7%)
No	167 (93%)
No. of intercostal spaces in IMN target	
2	1 (<1%)
3	105 (59%)
4	71 (40%)
5	2 (1%)

Methods and materials
179 breast cancer patients treated with adjuvant loco-regional radiotherapy including the IMN from 18 European departments were included in the study. Each department included 5 patients with left-sided and 5 patients with right-sided breast cancer. The prescription dose ranged from 39.9 Gy to 51.52 Gy in 15 to 28 fractions. Planning techniques included both 3D conformal and several inversely optimized techniques (see Table 1). If the clinically delivered treatment plan did not comply with defined target coverage requirements, the plan was modified retrospectively for this study until sufficient target coverage was reached. Sufficient target coverage was defined by the DBCG RT Committee as: V90% ≥ 95% of CTVn_IMN, V90% ≥ 95% of CTVn and V95% ≥ 95% of CTVp_breast/chest wall.

Results
40% of the treatment plans needed modification to fulfil the required dose for target coverage. Median mean heart dose was for left-sided breast cancer: 3.0 Gy (range, 1.1-8.2 Gy), for right-sided breast cancer 1.4 Gy (0.4-11.5 Gy), for left-sided breast cancer using breath-hold 2.8 Gy (1.1-7.4 Gy) and for left-sided breast cancer not using breath-hold: 5.2 Gy (2.2-8.2 Gy). Median mean dose to ipsilateral was 13.4 Gy (5.1-24.9 Gy) and median V17Gy/V20Gy (hypofractionated/normofractionated plans) for ipsilateral lung was 31% (0-57%).

To guide selection criteria for referral to proton therapy, the DBCG RT Committee chose to set cut-off points for dose to heart and lungs for departments that aimed for treating all patients with 3DCRT and in breath-hold, which 9 departments did (98% 3DCRT and 93% BH). We chose mean heart dose ≥ 4 Gy or lung V17Gy/V20Gy ≥ 37% as cut-off points for the proton therapy study based on dose-response relationships for ischemic heart disease and radiation pneumonitis in combination with capacity limitations for proton therapy. In the departments having 3DCRT and breath-hold as standard, 22% of the patients had a mean heart dose ≥ 4 Gy or lung V17Gy/V20Gy ≥ 37%. The remaining 9 departments mainly used inverse techniques (98%) where breath-hold was used in 31% of the patients. Fifty-two percent of these patients had a mean heart dose ≥ 4 Gy or lung V17Gy/V20Gy ≥ 37%.

Figure 5, ESTRO38 poster 2019

2.0 STUDY PLAN

This is a phase III clinically controlled randomised trial of proton versus photon therapy in early high-risk breast cancer patients selected on a high MHD and/or lung dose in the planning of photon-based RT.

Hypotheses:

Compared to photon RT, the risk of RT associated cardiac disease is lower using PT.

Compared to photon RT, the risk of RT-induced second cancer is reduced using PT.

Compared to photon RT, the risk of distant failure and death from breast cancer is reduced by using PT due to better dose coverage of targets including the IMN.

Aim: The primary aim is to investigate whether the prevalence of clinical cardiac events can be reduced by PT compared to photon RT in the treatment of breast cancer.

Primary endpoints

The cardiac diagnoses used for statistical considerations in this trial are those demonstrating significant association with left-sided breast cancer RT in a previous DBCG study on 19.464 women irradiated for breast cancer: ischaemic heart disease I20-25 and valvular heart disease I00-09, I01.0, I09.2, I34-39 (20). This is measured during follow up and reported at median 10 years after RT.

Secondary endpoints

Time to and location of recurrence, breast cancer survival, overall survival.

-Second cancer defined as cancer of the lung, contralateral breast, esophagus, thyroid gland and radiation associated sarcoma in the loco-regional region.
- Acute toxicity: acute toxicity scoring as in the DBCG Skagen trial 1 (60 patients with PT, 60 patients with photons).
- Late toxicity and patient satisfaction with cosmetic result: DBCG late toxicity scoring as in DBCG Skagen trial 1 including questions regarding rib fractures (all patients)
- Patient reported outcomes regarding loco-regional morbidities and socio-economic issues are measured as part of DCCL (DBCG Center & Clinic for Late Effects) (all patients)
- Translational research: Minimum one sample (at baseline) is collected from all patients treated in this trial (some patients will be invited to participate in a sub-study and provide 8 blood samples during the 10 year follow up). The baseline blood sample will be evaluated for cardiac and inflammatory markers of vascular damage (e.g.Troponins, Lymphocytes, Cholesterols). In addition, ^{15}O-water PET-CT scans are made in 50 patients from AUH to detect early vascular and pulmonary damage (before RT, at 1 and 3 years after RT). The PET-CT scans after RT will include whole lung in RT position to allow for deform co-registration with RT fields. Radiobiologic effects will be investigated at the end of spread-out Bragg Peak (21).

Choice of endpoints

The cardiac diagnoses used for statistical considerations in this trial are those demonstrating significant association with left-sided breast cancer RT in a previous DBCG study on 19.464 women irradiated for breast cancer: ischaemic heart disease I20-25 and valvular heart disease I00-09, I01.0, I09.2, I34-39 (20).

The DBCG has a long tradition for evaluating loco-regional morbidities following adjuvant RT. As of 2019, >4500 early breast cancer patients are in follow up after randomisation in four other DBCG trials (DBCG HYPO / PBI / Skagen Trial 1 & Natural trials), so to ensure coherence with earlier results, the DBCG scoring of loco-regional morbidities in this proton trial is mandatory both in the acute and late setting.

The acute morbidities include: radiation dermatitis, itching, pain, fatigue, dyspnea, cough, pneumonitis, dysphagia, increased sensation of tightness of the shoulder and lymphedema.

The late morbidities include: fibrosis, telangiectasia, edema of the breast & arm, shoulder function. Dyspigmentation is evaluated according the LENT-SOMA scoring scale, and cosmetic outcome is according to the Aaronson scale (22). Based on Harris´ 4-point scale the global cosmetic result after breast conservation is scored (23). Questionnaires for pain, swellings, discomfort, daily function and Body Image Scores are used (24-26). Also, we ask about lipo-injection, smoking, use of statins and fear of recurrence during follow up. In addition, rib fractures are reported, and these should be verified on imaging.

In 2020, the DCCL (DBCG Center & Clinic for Late Effects) launches a nationwide app-based follow up system to collect patient reported outcomes before, during and after therapy (funding obtained from the Danish Cancer Society, and Ethically approved by Region Midt 2019). The app uses international validated questionnaires to ask for socio-economic issues (education, employment, marital status), BMI, tobacco, alcohol, physical activity & function, comorbidity, general physical symptoms (loss of appetite, nausea, dyspnea, cough, pain, hot flushes, loco-regional sensibility disturbances), general symptoms & late effects (impaired concentration and memory, sleep disturbance, fatigue, depression, anxiety, impaired sexual life), work ability index, resources (emotional support and self-efficacy for managing symptoms). These data will be included as a secondary endpoint in the DBCG PROTON Trial.

Virtually all morbidities evaluated in this trial are using the same scales and scores as recommended in the European Particle Therapy Network (EPTN).

Follow-up program

Follow-up is for 10 years after RT. Acute toxicity during RT will be registered at DCPT (protons) or at the local centers (photons), and late toxicity at the local centers.

Acute toxicity: is evaluated at baseline, weekly during RT, 14-days post-treatment and thereafter every 2 weeks as long as there are symptoms above baseline level. Three and 6 months after end of RT the patients are contacted by telephone and evaluated for pneumonitis, i.e. if the patient is suspect for pneumonitis (has dry cough and dyspnea) a diagnostic CT thorax is recommended to verify the diagnosis.

Late toxicity: Baseline, year 1, 2, 3, 4, 5 and 10.

Both for acute and late toxicities, breast photos are mandatory (2 front photos and 2 side photos). Appendix 1.

Treatment details

Photon arm: Photon RT using DBCG guidelines for loco-regional RT including IMN RT to a total dose of 50 Gy/25 fractions, 5 fractions per week. Boost is accepted.

Proton arm: PT to a total dose of 50 Gy using a fixed Relative Biological Effectiveness (RBE) value of 1.1, 25 fractions, 5 fractions per week. Boost is accepted.

Patients can be part of other randomised trials, such as the DBCG Senomac trial, the DBCG Skagen trial 1, the DBCG RT Recon trial, the INDAX trial and the MASTER trial.

If the DBCG RT Committee changes the standard for dose and fractionation of loco-regional RT during the accrual of patients in the DBCG PROTON Trial, the new standard will also be used in this trial.

Independent Data Monitoring Committee (IDMC)

An IDMC will be established to monitor patient safety and treatment efficacy while the trial is ongoing. The IDMC will as minimum include an oncologist with extensive research record within breast RT and a biostatistician.

3.0 PATIENT SELECTION

Patients treated for invasive breast cancer or DCIS with indication for RT have photon RT plans made to fulfill the following: V95% ≥ 95% of CTVp_breast/chest wall, and if nodal RT is indicated V90% ≥ 95% of CTV_IMN and V90% ≥ 95% of CTVn. If these constraints lead to a MHD ≥4 Gy and/or V17/V20lung ≥ 37% the patient is a candidate for the DBCG PROTON Trial. It is not necessary to make a complete and treatable photon treatment plan before evaluating the heart/lung constraints. For patients <41 years old, there is a need for special focus on low dose to contralateral breast. For these young patients, the mean dose to the medial quadrants of the contralateral breast should be kept <1 Gy (19). If the patient accepts randomisation and is allocated photon RT, the photon RT plan will be modified according to standard DBCG guidelines based on patient and tumour characteristics. This will lead to compromises made on target coverage, such that the dose to heart and/or lung can be lowered.

Selection criteria

- Patient (female or male) ≥ 18 years who has a diagnosis of invasive breast cancer or DCIS pTis-4, pN0-N3, M0
- Synchronous bilateral breast cancer / DCIS is allowed
- The patient is eligible for adjuvant breast local or loco-regional RT.
- Mean heart dose is ≥4 Gy and / or V17/20 ipsilateral lung is ≥37% in a treatment plan based on sufficient target coverage as DBCG defined.
- Adjuvant systemic therapy is according to DBCG guidelines
- Boost (breast, chest wall and nodal), breast reconstruction (any type, except implants with metal), connective tissue disease, post-operative surgical complications, any breast size and seromas are allowed
- Patient with previous non-breast malignancy is accepted if the patient has been without disease minimum 5 years, and the treating oncologist estimates a low risk of recurrence.
- Life expectancy minimum 10 years

Exclusion criteria

- Previous breast cancer or DCIS of the breast
- Meta-chronous bilateral breast cancer (because the patient cannot be treated with both proton and photon therapy with respect to the primary endpoint (heart disease) and the secondary endpoint (tumor recurrence))
- Previous RT to the chest region
- Pregnant or lactating
- Conditions indicating that the patient cannot go through the RT or follow up
- Patients with Pacemaker or defibrillator are excluded until a guideline for handling them has been developed at the DCPT
- Unknown non-tissue implants upstream of the target volume. NB. all such non-tissue, non-metal objects must be delivered to the DCPT for stopping power determination and evaluation at least a week prior to radiation start.
- Metal implants in the radiation area, including metal in implants.

Type of randomisation

Included patients are randomised in a 1:1 ratio.

Stratification will be on institution, and on selection for the trial was due to heart or lung dose, and on local versus loco-regional RT. If the patient is a candidate for this trial both on heart and lung criteria, the patient will be put in the heart stratum during randomisation.

Statistical consideration

Primary endpoint
The primary endpoint of the trial is occurrence of cardiac events potentially attributable to radiotherapy. The cardiac diagnoses used for statistical considerations in this trial are those demonstrating significant association with left-sided breast cancer RT in a previous DBCG study on 19.464 women irradiated for breast cancer: ischaemic heart disease I20-25 and valvular heart disease I00-09, I01.0, I09.2, I34-39 (20).

The age distribution of breast cancer patients treated with loco-regional RT at OUH 2017-2018 showed a median age of 61 years and the distribution as follows

AGE	No. pts
30≤AGE<35	5
35≤AGE<40	4
40≤AGE<45	14
45≤AGE<50	10
50≤AGE<55	24
55≤AGE<60	19
60≤AGE<65	23
65≤AGE<70	23
70≤AGE<75	21
75≤AGE<80	19
80≤AGE<85	3
85≤AGE≤90	2

In the power calculations below we have assumed no patients above 80 to be conservative with respect to the expected event-rate on the primary endpoint.

LPR (the national patient register) indicates an age-weighted incidence of cardiac disease of 1180 per 100,000 life years in females.
We assume an average exposure of mean heart dose (MHD) 0.5 Gy in the proton arm and an average exposure of 4 Gy in the photon therapy arm. Assuming an excess relative risk of 20% per Gy MHD (Lorenzen et al, manuscript in preparation*)* we expect a 10% excess relative risk in the proton arm and 80% excess relative risk in the photon arm.

This leads to an estimated freedom from cardiac event rate in the background population after five years of an 'average' patient by weighted average of incidence rates:
FFHD_ref=(1-1180/100000)^5=94.2 %
FFHD_proton=(1-(1180*1.1)/100000)^5=93.7%
FFHD_photon=(1-(1180*1.8)/100000)^5=89.8%
The expected HR between the trial arms is thus HR=ln(0.937)/ln(0898)=0.605
The median time to event in the proton arm is estimated to m1=5*ln(0.5)/ln(0.937)=53.3 years
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The following parameters and assumptions are behind the power calculation:

Parameter	Parameter	Value	Background
Rate constant protons	λ_{Proton}	0.0131	5 year survival for primary endpoint= 93.7%
Rate constant photon	λ_{Photon}	0.0215	5 year survival for primary endpoint=89.8 %
Rate constant dropout	$\lambda_{recurrence}$	0.0349	5 year recurrence free rate=84% Censor on recurrence
Accrual time	6 years	250/year	Expected from Stick et al
Additional FU	3 years	N/A	3 years maturation

Significance level is 0.05 and power is 80% **with 2x751 patients accrued**.
The total number of patients accrued is therefore 1502 patients, whereafter the accrual closes.

Main secondary endpoint
Potential loss of disease control due to target compromises is the main secondary endpoint to control. At present, there is no clinical data to elucidate the detrimental effect of partial target coverage. Below we present statistical power for assumed hazard ratios and corresponding absolute excess risks to illustrate the statistical power to detect clinically relevant loss of disease control in the photon arm due to compromises in target coverage. For reference, a 4 percentage points absolute risk difference was deemed to be the limit of clinical relevance under the design considerations for the Poortmans trial and the HR for distant recurrence in Poortmans/DBCG IMN is approximately HR=0.86 and HR=0.89, respectively (2,3)
All calculations are made assuming a reference 5-year free from distant recurrence of 84% at 5 years in the photon arm and a type 1 error probability of 5%. HR are proton vs. photon. Power is for the sample size of 2*751 subjects as described in primary endpoint and analyzed at the same time (6+3 years).

Assumed HR	Assumed photon 5 year free from distant recurrence	Corresponding proton 5 year free from distant recurrence	Difference	Power to detect
0.86	84%	86%	2%	23%
0.80	84%	87%	3%	43%
0.75	84%	88%	4%	61%
0.7	84%	88.5%	4.5%	**78%**
0.65	84%	89.3%	5.3%	**90%**

In other words, we are likely to deem protons superior in terms of tumor control (or, equivalently, target compromises on photons detrimental) if the true absolute risk difference is approximately 4.5% or greater.

Interim reports

Interim reports with statistical analyses will be prepared and discussed with the IDMC at median year 1, 3 and 5 until the primary analysis has been submitted. In general, the interim reports will contain information about patient accrual rate with a projected completion date for the accrual phase, data quality, compliance rate of treatment delivery with the protocol distributions of important prognostic baseline variables. The IDMC can request further data. The primary data analysis is planned at median 10 years follow up. Further analyses will be consulted with the IDMC.

Statistical analysis

The following statistical analyses will be applied:

The primary endpoints will be analysed by the Kaplan-Meier method with time to occurrence of the first event of cardiac disease and using log-rank test for comparison. Further, 2x2 contingency tables of 10-year incidences will be supplied, supported by fishers exact test for difference.

The mean changes in questionnaires about patient reported outcome measures will be compared by two-sample t tests. The clinically relevant differences in the measures depend on each item, and we will evaluate the results in harmony with the same endpoints in previous DBCG trials. Actuarial rate of overall survival, breast cancer survival and loco-regional recurrences will be reported. Cox proportional hazards regression models will be used for multivariate analyses of survival-related endpoints.

Data collection and management

The DBCG database will serve as the central database regarding patient, tumour and treatment characteristics in this trial. An independent database for collection of RT related breast photos will be established at Experimental Clinical Oncology, AUH.

All RT plans (both proton and photon plans) will be submitted to the Danish National Dose Plan Bank, which is approved for clinical trials.

Data will be entered only by investigators or individuals authorized by the investigators. Data entry is online from all Danish breast cancer RT centres, and a study specific interface will be added for the present trial with the necessary CRFs, protocol parameters, questionnaires, morbidity and outcome registrations. Two Factor Authentification is used.

A Data Management Plan (DMP) will outline the necessary requirements of data collection and management, including how data will be stored and analysed. The DMP will be based on the obligations and requirements from the Danish Data Protection Agency, The Act on Processing of Personal data (lov om behandling af personoplysninger) and the National Research Ethics Committee. No approvals have been obtained by Dec, 2019.

All authorities who need to access data by law will be permitted data access to the database. If other research groups wish to access any of the data, they may contact the principal investigator for data agreement, however, by completion of the trial data will be made available to other researchers in anonymized form through the Zenodo open data repository. The trial will be registered at ClinicalTrials.gov. The trial will be conducted according to the principles of Good Clinical Practice.

Inclusion procedure

The inclusion procedure is through the database of DBCG. It is an online system allowing the treating staff to perform the inclusion procedure within few minutes. The randomisation is stratified by institution, high heart or lung dose, and local /loco-regional RT.

Information of the patient

All patients being candidates for adjuvant breast radiation therapy are invited to have information regarding this therapy in the Department of Oncology, and the invitation encourages the patient to bring along an assessor to the information. The meeting takes place in a quiet and undisturbed room. The informing doctor first informs the patient about the standard photon therapy. It is mentioned that relatively few patients may be candidates for proton therapy, thus proton therapy is unlikely to be relevant in patients treated with breast only RT, but if the patient is a candidate for loco-regional RT the likelihood is around 20%. The patient is informed that it may occur that this will happen to her/him, and in that case the department will contact the patient for further information after the planning CT scan is evaluated. After the initial consultation, the patient has a planning CT scan (either later the same day or few days later). During planning of the photon radiation therapy it will become clear that around 20% of all

patients having loco-regional RT may be candidates for proton therapy. Patients with MHD ≥4 Gy and/or V17/V20 lung ≥37% are contacted by phone and/or letter and invited for a new meeting in the clinic, and the invitation encourages the patient to bring along an assessor to the information. Here the patient is invited to accept randomisation in the proton therapy trial with the potential that proton therapy lowers the dose to heart / lung and potentially improves target dose coverage thereby improving prognosis. The patient is handed a written information about the proton phase III trial. The patient is thereafter invited to come to another session at the hospital to give consent to participate in the trial and be randomised to have photon versus proton therapy, if that is what he/she wants. After his/her written consent, baseline morbidity evaluation is performed, and the patient is informed as soon as possible about the practical issues related to having proton therapy at DCPT.
At the consultation where the patient is informed that he/she is a candidate for proton therapy and in the written information about the randomised trial the patient is informed that we want information from his/her patient file passed on to the study regarding tumour characteristics, and also about recurrence and survival status for 15 years after radiation therapy was delivered. If he/she has a recurrence we need data passed on from his/her patient file regarding histopathological type and localisation of the recurrence and when the recurrence took place. The tumour characteristics are: tumour histological type, size, lymph node status (how many removed and how many with metastasis and what type of metastasis), malignancy grade/ER/PR/HER2/Ki67 status, Q score, breast cancer subtype, genome expression profiles including also BRCA status if available, resection margin, surgical procedure. Also information about serious events like a new cancer, heart disease, lung disease and stroke is passed on from his/her patient file, because these events may be related to the radiation therapy. If an increased risk of any of these events is associated with proton therapy, it will influence the decision on what is to be future standard therapy. In addition, the patient accepts that information/data reported from the patient through the DCCL app is passed on to the study group of the DBCG PROTON Trial. These data concerns tumour, treatment, socio-economic and psychological items of interest which may influence or be influenced by the course of a breast cancer.
If the patient declines enrolment into the randomised trial, proton therapy cannot be provided to the patient, and standard photon therapy is given according to DBCG guidelines.

Here is a suggestion for patient flow before start of treatment at DCPT:

The patient has no chemotherapy

Surgery → First consultation in Dept Oncology → planning CT → candidate for proton trial (RT plan does not need to be final) → telephone call to patient with early information about proton trial and/or a letter is sent to the patient → as soon as possible new consultation in Dept Oncology with trial proposal → randomization same day or as soon as possible → referral to DCPT → first consultation at DCPT within 6 days. Treatment starts as soon as possible.

The patient has chemotherapy

Surgery → starts chemotherapy → planning CT before chemotherapy course 5 (if 6 series are planned, otherwise before course 7 if 8 series are planned) → candidate for proton trial (RT plan does not need to be final) → telephone call to patient with early information about proton trial and/or a letter is sent to the patient → as soon as possible new consultation in Dept Oncology with trial proposal → randomization same day or as soon as possible → referral to DCPT → first consultation at DCPT within 6 days or after end of chemotherapy. Treatment starts as soon as possible, but not earlier than 2 weeks after last chemotherapy was given.

If the patient is referred to DCPT for therapy, the referring hospital will inform the patient about hotel and reimbursement of travel and other costs during the stay at DCPT. The guideline for this is dependent on the referring hospital and geography of the patient, and each Dept Oncology in Denmark has an expert/office who can inform the patient on an individual level.

Risk to the patient

Patients included in this randomised trial do not receive more radiation than they would

otherwise, except a new planning CT scan is needed for proton therapy planning. It is, however, expected that proton therapy will improve dose to targets and lower the dose to risk organs. The biologically effective dose (BED) calculated for proton therapy is based on the photon therapy, and the proton therapy will provide the same dose as photon therapy. For patients treated with a boost (sequential or as a simultaneously integrated boost (SIB)) the boost therapy is calculated and chosen to resemble as much as possible the sequential boost doses. These doses are currently being investigated as part of the DBCG RT Skagen trial 1.
All patients treated with protons will have daily conebeam CT (CBCT) for set-up verification. Some Danish departments already use CBCT for daily set-up of all irradiated patients including breast cancer patients (Vejle and Odense), and it is expected that the majority of the other participating departments start using routinely CBCT for daily set-up during the time of accrual in the DBCG PROTON trial. The accelerators in Vejle and Odense are from Elekta, and the CBCT made by Elekta has a field of view, which can include the chest and nodal regions. The other Danish departments have Varian accelerators, and the CBCT technique on those machines are not yet optimal for scanning both chest and nodal volumes. Varian is working on improving their CBCT technique, and it is likely that CBCT will become daily standard for set-up when their new CBCT technique is launched. Set-up verification without CBCT is usually including kV and MV pictures, which also give a small dose to the patient, but the different departments across DK use different low-dose set-up scans, and these may vary over time. A CBCT causes 1.2 mSv, and as an example the daily set-up pictures used routinely in Aarhus cause 0.1 mSv. The radiobiological effect of 1 Sv (Sievert) is estimated to be 1 Gy (Gray). The worst case scenario is a patient treated with 63 Gy in 28 fractions, thus in principle such a patient has 28 daily conebeam CT scans, total dose from that is 28x1.2 mSv=33.6 mSv. Thus, the patient has a total dose 63 Gy+0.0336 Gy=63,0336 Gy, thus the increase in total dose from conebeam CT is 0.053%, which is considered acceptable. The purpose of the CBCT is to ensure that the patient position is optimal, and it provides the best possibility to detect, if unanticipated anatomical changes have occurred.
It is expected that there will be no difference in the radiation induced late morbidities except a lower risk of heart and lung radiation induced morbidities due to lower doses to these organs at risk using protons. The discomfort for the patient during therapy is expected identical, except many patients living far from DCPT will need to stay at a hotel during the weeks of therapy. The cost for the hotel stay is covered by the Danish Health Authorities. Despite the need for extra CT scans for patients treated with proton therapy, there is no doubt that the total integral dose of radiation is lower at end of therapy for patients treated with protons compared with photons.

If the patient regrets his/her consent to the study he/she can withdraw the consent at any time, and he/she will then be treated according to the standard DBCG guidelines. He/She will be informed about this during the first information about the study and also in writing. If the patient withdraws the consent during radiation therapy, he/she will be treated with photons for the rest of the therapy, and the number of fractions then depends on how much dose was already received.

Conclusion

Through this trial proton therapy will be introduced in Denmark in a controlled manner with special focus on potentially life-threatening late radiation-induced morbidity. Patients will be followed prospectively with regard to loco-regional morbidity evaluation for 10 years after radiation therapy, and any deviation from the expected course of development of morbidity will be monitored and reported. During 2020, the DBCG will launch a nationwide app-based follow up system, where all Danish breast cancer patients are followed from before surgery until they no longer want to answer yearly questions. Through that app the DBCG PROTON Trial will collect PROM including morbidities, comorbidities, habits of tobacco/alcohol, data related to socio-economic and general symptoms and resources. The DBCG PROTON Trial is supported and has participation from all departments in the DBCG RT committee, and consequences of the study for Danish patients will be drawn in the DBCG RT committee. The inclusion criteria and the primary and secondary endpoints of the DBCG PROTON Trial are copied by other members of the Skagen Meeting Group, thus there will be an overlap with the planned randomised proton therapy trials in UK, F and N, and the trial will potentially be able to validate the Dutch NTCP (normal tissue complication probability) model-based strategy. Thus, the

DBCG PROTON Trial has potential for meta-analyses with at least 3 other countries´s proton trials for breast cancer.

4.0 RADIATION THERAPY

Planning CT scan and target volumes

Planning CT scan
The patient is scanned in supine treatment position with one or both arms abducted about 120˚. In selected cases or as part of a later defined study, it is accepted to do the planning CT scan while the patient has one/both arms in other positions. The head is positioned straight with the chin slightly upwards or a little tilted to the contralateral side, avoiding skin folds at the level of the lower neck. Fixation for proton therapy is done according to the guidelines at the DCPT, and a daily reproducibility of approx. 5 mm must be achieved. The scanned volume is neck and breast region including both lungs. The slice thickness is max 3mm. Respiratory gated techniques are generally recommended for photon treatment, but for proton treatment it may not be needed, however, it may be used in selected patients if deemed necessary by the specialists at the DCPT.

Breast CTV and lymph node targets

The CTV lymph node targets are delineated according to the ESTRO consensus guidelines (27-29), and the structure names must follow the nomenclature of the ESTRO consensus, Table 2. It is not acceptable to delineate all the CTVn´s as one large volume. Regarding dose coverage of the CTVn_IMN, it is recommended to include intercostal levels 1, 2, 3 in all patients, and in patients with a tumour localization in the lower-inner quadrant of the breast the IMN level 4 is also recommended included in the target. For all patients referred to the DCPT, the DCPT must have access to the full surgical and pathology reports and all diagnostic imaging related to the breast cancer to ensure the relevant targets are irradiated.

Table 2, List of nomenclature to be used in this trial according to the ESTRO and DBCG guidelines. *Only for patients <50 years at breast surgery

Name of target
CTVn_L1
CTVn_L2
CTVn_L3
CTVn_L4
CTVn_IMN
CTVn_interpect
CTVn_boost 1, 2, 3 etc
CTVp_breast
CTVp_chestwall
CTVp_tumourbed
CTVp_boost
PRV_contralat breast
PRV_contra_cranial*
PRV_contra_caudal*
Heart
A_LADCoronary
Lung_ipsilat
HumeralHead
Lung_contralat
PRV_Humeral head
Thyroid
Esophagus
Trachea

Boost CTV

Tumour bed should be delineated based on all available information from pre-operative imaging, surgical report, pathology report and the localization of the surgical clips. The clips must be positioned according to a protocol, and the radiation oncologist must be aware of this protocol. If oncoplastic surgery has been carried out, a close collaboration between the surgeon and the oncologist is particularly important if the patient is a candidate for a boost. The CTVp_boost is generated by adding a 5mm margin to the tumour bed inside the CTV breast. Notice, that nodal boost is allowed. If several nodal boost volumes are in play, they are named CTVn_boost 1, 2, 3 etc.

Contralateral breast

In all patients the contralateral breast is delineated. For patients <50 years on the day of breast surgery, the 2 medial quadrants are also delineated to allow dose calculation. The nipple defines medio-lateral and cranio-caudal orientation for definition of PRV_contra_cranial and PRV_contra_caudal.

Lung and heart

Delineation of lung, heart and LADCA is according to DBCG guidelines (30, 31). As part of research projects all cardiac sub-volumes will be automated delineated when submitted to the National Dose Plan Bank.

Breast implants

Patients included in this trial may have breast implants in the treated breast. In Denmark, permanent implants and expander implants are from the company Mentor, and both types of implants have a textured surface. The permanent silicone implant is named CPG Mentor, and the expander implant is named CPX 4. Both types of implants have been tested at DCPT, but only implants without metal are accepted for proton therapy. Other types of implants without metal are only accepted if the specific implant has been tested at DCPT before proton therapy is initiated. In such cases, the DCPT is contacted immediately to make sure the patient can be accepted (ideally before informing the patient).

Thyroid gland, esophagus and trachea

The thyroid gland is delineated according to Dahanca.dk guidelines. The trachea is delineated from the caudal edge of the cricoid cartilage and in caudal direction to 20 mm cranial to the bifurcation (where the trachea splits into bronchus). The esophagus is delineated in all slices where trachea is present (thus not to the ventricle), which is also the guideline in DOLG (Danish Lung Cancer Group).

Planning target volume (PTV)

PTV is generated by adding a margin around the CTV to account for set up errors during therapy. When the lymph nodes are target, a PTVn including all the CTVn´s is defined. Correspondingly a PTVp_breast/chest wall is generated from CTVp_breast/chest wall. In general PTV should be cropped to 5 mm beneath the skin. A typical CTV to PTV margin is 5mm in all directions. However the actual setup error depends on immobilisation of the patient and on the image guidance strategy, and therefore varies among departments. Each department should perform measurements to determine their CTV to PTV margin. The PTV_boost is typically defined as the CTV_boost + 5mm margin in all directions. For proton therapy, the PTV can be replaced by robust optimisation where the parameters are defined by the center specific setup uncertainty and stopping power range uncertainty.

Dosimetry and organs at risk

Treatment planning photon

Treatment planning is based on the ICRU 50, 62 and 83 recommendations (32). Dose is prescribed and dosed in CTV as per DBCG guidelines. For 3D-CRT planning it is recommended to use a technique based on a single isocenter with tangential fields with parallel posterior field edges to cover PTVp_breast / PTVp_chest wall. A 25mm skinflash is applied to account for potential swelling of the breast/chest wall. In case of simultaneous integrated boost, fields are

added to cover the PTV_boost. For regional nodes radiation therapy an anterior periclavicular field is used, and an opposing posterior field may be added to ensure homogeneous dose in the target. Wedges and electronic compensation may be used to obtain dose homogeneity. It is accepted to use intensity-modulated RT techniques including VMAT and Tomotherapy. A bolus on the lumpectomy scar is not accepted as routine, but may be indicated in special cases, whilst a bolus on the mastectomy scar in general is accepted (in 2019, DBCG guideline is to use bolus along the mastectomy scar in cases with T3 breast cancer).
CTVp (breast/chestwall) is to be covered with doses of 95-107% if therapy is normofractionated, and 95-105% if therapy is hypofractionated. The volume of CTVp receiving 107%<dose≤110% (normofractionated) or 105%<dose≤108% (hypofractionated) must be <2%. The overdosage should preferably be distributed over several areas.
CTVn's are to be covered with doses 90-107% with $D_{2\%}$≤ 108% (normofractionated therapy) and 90-105% with $D_{2\%}$≤ 106% (hypofractionated therapy). No volume except in the build-up zones must in principle receive dose <95%. The maximum dose to the treated volume should be kept below 108% (hypofractionated therapy) / 110% (normofractionated therapy). Photon energy is chosen to fulfil 95% dose 5 mm under the skin surface. Multi-leaf collimation is used to minimise the risk of dose to organs at risk.
Dose calculation must be based on modern dose algorithms (Monte Carlo, AAA, Collapsed Cone or similar) with inhomogeneity correction.
In few patients the CTV boost is positioned at the border between the breast and the regional nodes. In these rare occasions it must be avoided that the field edges overlap in the area of the CTV boost due to the risk of triple trouble in the hypofractionated area in particular.

Treatment planning proton
The first Danish breast cancer patient received proton therapy as of October 2019, thus at the current time (December 2019) much work is going on regarding the details for proton treatment planning. All patients will be positioned in supine position with one/both arms elevated, and target volume definition is according to ESTROs guidelines. Dose, fractionation and boost follows DBCG guidelines and using a fixed RBE of 1.1. The field arrangement will in most patients be one or a few *en face* fields, however, the exact strategy will depend on a treatment protocol, which is expected to be modified as more experience is gained. Depending on the individual anatomy, there may be a part of the chest wall in the upper inner quadrant which is not included in a target defined according to the ESTRO consensus for target volume delineation. Thus, there may be a "hole" in the target, which is NOT to be covered with dose, because it is not considered target. The staff at DCPT has a close collaboration with international colleagues already treating breast cancer patients with protons. The treatment protocol with modifications will be published as a technical report in the initial phase of patient accrual thus increasing the level of knowledge regarding this new treatment technique.
To ensure optimal robustness, a weekly planning CT scan is likely necessary at least in the initial phase of the trial. The need for extra CT scans will follow the DCPT guidelines.

Doses
Patients treated with 50 Gy / 25 fractions are treated with 2.00 Gy per fraction, 5 days weekly, to breast or chestwall and regional nodes. Patients treated with 40 Gy / 15 fractions are treated with 2.67 Gy per fraction, 5 days weekly, to breast or chest wall and regional nodes. If the patient is a candidate for boost either to tumour bed after lumpectomy and due to a non-radical surgical procedure, the boost will be provided as a simultaneous integrated boost with the doses listed in Table 3.
In 2020/2021 results from a randomised phase III trial conducted in UK may demonstrate 26 Gy/5fr, 5 fr per week, as feasible for adjuvant breast RT. This fractionation schedule may be accepted by the DBCG RT Committee for selected Danish patients inside a future DBCG trial, which potentially can also include patients treated with proton therapy. The DBCG RT Committee will decide if this is acceptable.

Table 3
Doses for simultaneous integrated boost and non-boost areas in patients treated with either 50 Gy / 25 fr or 40 Gy / 15 fr and with an indication for boost. The boost levels in the first column (Boost) indicate the different options in play according to DBCG guidelines.

Boost	SIB	Boost dose / fraction	Breast/chestwall	Non-boost dose/fraction	Fract
50Gy+10Gy	57 Gy	2.28 Gy	50 Gy	2.00 Gy	25
50Gy+16Gy	63 Gy	2.25 Gy	51.52 Gy	1.84 Gy	28
40Gy+10Gy	45.75 Gy	3.05 Gy	40 Gy	2.67 Gy	15
40Gy+16 Gy	52.2 Gy	2.90 Gy	42.3 Gy	2.35 Gy	18

Organs at risk

Organs at risk are the heart, LADCA, lung, chest wall, ribs and contralateral breast, and in the nodal areas it is the medulla spinalis, brachial plexus, the shoulder joint and the vessels. DBCG guidelines for therapy with 50 Gy / 25 fractions defines that max 5% of the heart may receive max 40 Gy, and that max 10% of the heart may receive max 20 Gy. The LADCA should be delineated and may max receive 17 Gy (as point dose). For the ipsilateral lung the DBCG guideline defines that max 35% of the lung may receive 20 Gy, and that max mean ipsilateral lung dose is 18 Gy. Contralateral breast should receive as little dose as possible. If the patient is <41 years old, the mean dose to medial breast quadrants in contralateral breast must be max 1 Gy (19).

The risk of radiation induced brachial plexopathy is closely related to dose per fraction and total dose (33). In the 1950s, the use of 60 Gy total dose to regional nodes and 5 Gy / fraction caused plexopathy in 66% of patients. In the 1960s therapy was 45-50 Gy using 4 Gy / fraction and patient movement between each radiation field (because the gantry was fixed) resulting in overlapping doses caused plexopathy in 50% of patients. In the 1970s-1980s therapy was based on 45-50 Gy using 3 Gy / fraction resulting in 10-15% patients with plexopathy. The incidence of brachial plexopathy is today <1-2% in patients receiving plexus total doses <55 Gy in 2 Gy/fraction. The DBCG guideline recommends a max dose in the brachial plexus of 54 Gy (2 Gy/fraction) (Table 4). Using an α/β 2 Gy, this corresponds to a BED of 108 Gy, so if therapy was based on 2.67 Gy/fraction a max dose of 46.25 Gy has the same BED (=108 Gy). Thus a dose of 40 Gy/15 fractions is expected to be acceptable.

The shoulder joint and the connective tissues around it should receive as low a dose as possible, and preferably less than 50% dose.

The constraints for organs at risk in this trial will follow the DBCG guidelines, and if changes are made in the DBCG guidelines during the course of this trial, these changes will also be implemented and followed by this trial.

The criteria mentioned above are to be fulfilled, however, there may be situations where the constraints cannot be met unless serious under-dosing of the cancer target is done, so it is important to always balance gain and risk for the individual cancer. Distant failure from poor RT of the IMN (or other nodal targets) may kill the patient.

Table 4
Overview of maximal accepted doses according to randomization arm.

OAR	V17 (hypo)	V20 (normo)	V35 (hypo)	V40 (normo)	Max dose
Heart	10%	10%	5%	5%	
Ipsilat lung	35%	35%	-	-	Mean max 18 Gy
Brachial plexus					54 Gy (normo) 46.25 Gy (hypo)
Medulla spinalis					45 Gy (normo) 38.54 Gy (hypo)

Priority photons:

The highest priority is given to the tumour bed irrespective of breast conservation or mastectomy. Thereafter priority should be given to the internal mammary nodes based on

recent data from the DBCG IMN study. Fields should be arranged in a way to lower the dose to heart and lung, and therefore compromises may be accepted on CTVp_breast or CTVp_chest wall. Lower priority is given to PTV provided that the compromise is distant from the tumor bed, and finally contralateral breast has priority.

The balance between dose coverage of the CTVn_IMN versus the LADCA/heart should be evaluated based on the individual patient characteristics and technical aspects.
The above mentioned accepted doses for organs at risk are not to be considered safety doses. At any time it is important to strive to achieve as low doses in the organs at risk whilst assuring sufficient doses to the targets. If it is chosen to violate the ICRU recommendations when approving the dose plan focus should be on avoiding *double trouble*.

Verification of the radiation therapy

The DCPT will use its own routine system for verification of the radiation therapy, most likely daily conebeam CT scans. The system for verification will be used in all patients. Patients treated with photons have treatment verification according to institutional guidelines, however, with conebeam CT scans at least every 5 treatments to allow for investigations of robustness. Thus, conebeam CT scans are made at fraction 1, 6, 11, 16, and 21, and at 26 if the patient has 28 fractions. The conebeam CT must include the level of the heart.

Quality assurance of the radiation therapy

Before start of the randomised trial all participating Danish departments will be informed about the trial protocol. Principal investigator is responsible for that.
For participating centres outside Denmark detailed information about the protocol and treatment planning is assured by the principal investigator either visiting the centre abroad or by people from the centre abroad visiting the DCPT in Aarhus.
All proton and photon therapy plans from patients treated as part of the trial must be submitted to the Danish national dose plan bank and detailed quality assurance based on a protocol for QA will be performed based on these plans. For other details, please read the paragraph “5.0 National dose plan bank”.

Postponement of therapy and treatment breaks

Postponement of radiation therapy must be as seldom as possible. Treatment breaks must be as short as possible. Since the radiation therapy in this study is adjuvant it is in general not indicated to compensate for lost fractions, thus when the patient is ready to resume therapy after a break, she/he will continue the radiation plan until all fractions planned are given.

5.0 NATIONAL DOSE PLAN BANK

All treatment plans for patients treated in this protocol must be submitted to the Danish national dose plan bank for quality assurance and detailed research. As part of later planned research studies more detailed volumes for example of the heart will be delineated. It is of utmost importance that the delineated structures are named according to the nomenclature used in the ESTRO target consensus (Table 2), and the local trial investigator is responsible for this. All details about the treatment planning will be collected through the dose plan bank, thus there will be no reporting of doses to organs at risk outside the dose plan bank. For patients treated outside Denmark as part of this trial treatment plans must also be submitted to the Danish national dose plan bank. The randomisation number will serve a patient identifier.
The submission must take place prospectively with max 2 months intervals. This is to assure access to plans for quality assurance. All images related to planning and treatment verification is also collected in the bank.

6.0. EVALUATION OF RADIATION ASSOCIATED MORBIDITY

Table 5 is an overview of the follow up for patients included in the trial.

Cardiac morbidity

The primary endpoint is the cumulative incidence of ischaemic heart disease and valvular disease at 10 years after inclusion in the trial. These endpoints are chosen based on previous Danish studies, and are to be collected from the Danish National Registry of Diseases (20). These diagnoses reflect clinically evident heart disease. However, since non-Danish departments may also participate in the trial, it is necessary to collect cardiac disease events prospectively during follow up in the trial. At each follow up visit, the patient is asked to report cardiac disease (including hypertension) including date of the event. Cardiac disease must be reported with an ICD10 code (e.g. www.diagnosekoder.dk). Cardiac events other than ischaemic heart disease and valvular disease constitute a secondary endpoint.
During the yearly follow up visit the following information is registered: height, weight, blood pressure, diabetes (irrespective type 1 or 2), known hypertension, known dyslipidmia, known familiar heart disease (previous AMI or by-pass surgery in parents or siblings at age <55 yr if male and <65 yr in females).

Cancer related endpoints

Cancer related endpoints are secondary endpoints in this randomised trial, and they are local recurrence, regional recurrence, distant metastasis, disease-specific survival and overall survival. Ipsilateral local recurrence is defined as any tumour in the breast or skin over the breast or chest wall. A detailed reporting on the localisation of the local recurrence will be provided through evaluation among the oncologist, the pathologist, the radiologist and the surgeon. Deciding whether a recurrence is a true recurrence or a new primary depends on the tumour-biological tests made by the pathologist according to current guidelines at the treating hospital. Regional recurrence is defined as tumour in ipsilateral axilla level 1, 2, 3, 4, IMN or in the interpectoral nodes. Metastases other places in the body are distant metastases. Metastases will be identified by a combination of clinical, haematological, radiological and histopathological evaluations. There may be clinical situations where histopathological evaluation is not feasible or clinically meaningful, and the oncologist will then decide whether or not the patient has a recurrence.

	Baseline	At last RT fraction	1 year	2 years	3 years	4 years	5 years	10 years
Loco-regional morbidity	x		x	x	x	x	x	x
Patient characteristics	x		x	x	x	x	x	X
Tumour and treatment characteristics	x		x	x	x	x	x	X
Recurrence pattern, new malignancy	x		x	x	x	x	x	X
RT QA	x	x						
Patient and societal cost	x	x	x	x	x	x	x	x
Blood	x	x	x	x	x	x	x	x
Blood, 200 pts*		x	x	x	x	x	x	x
Acute radiation morbidity, 120 pts	x							
^{15}O-H_2O-PET, 50 pts	x		x		x			
Cardiac CT and ECCO, 150 pts	x		x		x		x	x

Table 5
Patient course from randomisation to end of follow up in the DBCG PROTON Trial.
Measurements/evaluations from all patients are highlighted in grey
Measurements/evaluations from subgroups of patients are not highlighted
*Notice, that the 200 blood samples are taken from patients included in the PET CT study (N=50 patients) or in the cardiac CT and ECCO study (N=150 patients). The blood samples in these 2 substudies and all baseline blood samples from AUH are 50 ml. All other blood samples are 30 ml to be stored in the Danish cancer Biobank.

Explanation of the content of the investigations performed during follow up
Loco-regional morbidity (breast induration, dyspigmentation, telangiectasia, breast edema, arm-shoulder function, arm edema, cosmetic score, patient satisfaction, patient functioning, photos). Body Image Scale is used.
Patient characteristics: weight, height, tobacco, comorbidity including cardiac disease (ICD10 scores), use of statins including generic name, all measurements from the *DCCL app* (PROMS regarding physical, psychological and socio-economical scores). Results (except cardiac disease) may be published at 3, 5 and 10 years median follow up.
Tumour and treatment characteristics: histopathology, surgical type, adherence to systemic therapy from baseline and during follow up (type and date).
Recurrence pattern (local, regional and distant failures, overall survival) and other malignancies. Analysis at 5 and 10 yr.
RT QA: Radiation Therapy Quality Assurance. Investigations regarding robustness of proton versus photon radiation therapy (subgroups of lumpectomy, mastectomy, reconstruction, large/small volumes, seromas, lymphedema and other surgical/therapy related complications). Treatment plans and any imaging carried out as part of the therapy of the patient may be used for this research. Publications when 100 patients or more are available in relevant groups.
Patient and societal cost, value to patient/society, health outcome that matters to the patient, cost, incremental cost effectiveness. Feasibility of PT (patient accept of PT, travel/housing issues, adherence to PT, patient satisfaction, geographical differences).
Blood sample: It is part of this trial to have one blood sample taken at baseline as part of the patient's treatment. This blood sample is investigated at a later time point The blood samples must be stored in a freezer within 2 hours or if longer time is expected taken in Strecktubes or other similar blood collection tubes allowing detection of high-quality cell-free DNA. The blood sample is collected and stored in the Danish Cancer Biobank, except some of the blood samples taken at Aarhus University Hospital are stored in a biobank established as part of the DBCG proton trial at Experimental Clinical Oncology, Department of Oncology, Aarhus University Hospital. The blood samples for the Danish Cancer Biobank is 30 ml, and for the samples stored at AUH it will be 20 mL plasma and 20 mL serum samples (see below).
The blood samples stored in the Danish Cancer Biobank will be collected prior to start of radiation therapy under the auspices of Danish Cancer Biobank (DCB)/Regionernes Bio- og genombank (RBGB) and stored in this clinical biobank. When our study requests the blood sample from DCB/RBGB, we will establish a research biobank where some of the collected blood samples will be transferred to. Some of the blood samples will remain in the clinical biobank (DCB/RBGB) for the patient's treatment or for other studies to use if the relevant approvals are obtained. All biological material will be destroyed in the research biobank at the latest 10 years after the last patient entered the trial.

The costs for blood samples vary among Danish departments, thus some departments may choose to only collect a baseline blood sample (small package, see below), whilst others may decide to collect more samples (large package). The departments will decide which approach they prefer before start of the trial. The majority of samples in this trial will be stored as 30 ml samples in the Danish Cancer Biobank. A subgroup of 200 patients accrued at AUH will have 50 ml samples collected, because they participate in substudies, where extra analyses are relevant.

Here is an overview of the blood samples:
All patients (small package): Baseline blood sample 30 ml, except patients accrued at AUH have a 50 ml sample.
For departments who can afford extra samples (large package): 30 ml blood sample at last radiation therapy visit and at follow up year 1, 2, 3, 4, 5 and 10.
At AUH: All patients have 50 ml baseline sample, and the 200 patients included in the 2 substudies (the PET CT study (N=50 patients) or in the cardiac CT and ECCO study (N=150 patients) have 50 ml samples at same time slots as in the large package. All other blood samples collected at AUH will be 30 ml samples for the Danish Cancer Biobank.

The 30 ml samples for the Danish Cancer Biobank are stored as: 1x9ml EDTA in fractions of 1.5 ml whole blood, 2x2 ml plasma and 1 buffy coat. 2x9 ml serum in fractions of 4x2 ml serum.

Translational research

Blood samples: Serial blood samples are collected from patients accrued in departments who can afford the "large package" of blood samples and from patients participating in the ^{15}O-water-PET CT study (50 patients) or in the Cardiac CT and Echocardiography study (150 patients),. When our study requests the blood sample from DCB/RBGB, we will establish a research biobank where some of the collected blood samples will be transferred to and translational analyses for research purpose are carried out. The evaluations will include measurement of troponins, pro-BNP, cholesterols, markers of radiation response, heart and lung disease and other markers deemed relevant at a later point of time. The blood samples must be stored in a freezer within 2 hours or taken in Strecktubes or other similar blood collection tubes allowing detection of high-quality cell-free DNA. The samples are 30 ml if stored in the Danish Cancer Biobank and the samples from AUH will contain 20 mL plasma and 20 mL serum.
No extra consent from the patient is needed for analyses of the blood samples.
Acute RT morbidity (0-6 months) in the first 120 patients (60 patients with proton therapy and 60 patients with photon therapy) (Table 6). Only patients who consent for this substudy are analysed.
^{15}O-water-PET CT Cardiac morbidity evaluated with ^{15}O-water-PET CT at baseline, and after 1 and 3 years. 50 patients from Aarhus University Hospital. Only patients who consent for this substudy are analysed.
Cardiac CT and Echocardiography: Echocardiography and CT heart modified to include whole lung in 150 patients from Aarhus University Hospital. Only patients who consent for this substudy are analysed.

Morbidity related endpoints

In this trial detailed evaluation of acute and late radiation-induced morbidity is a key factor and late morbidity evaluation will take place in all patients. Table 6 illustrates what and when evaluation of acute late radiation-induced morbidity is to take place. Detailed acute morbidity evaluation based on photon loco-regional radiation therapy has been collected in the DBCG Skagen Trial 1, thus acute morbidity in this trial will be performed in 60 patients treated with protons and 60 patients treated with photons. It is emphasized that at any time a late radiation-related morbidity is detected a full morbidity evaluation must be performed. Charlson´s comorbidity index is registered at the Danish surgical departments. For participating departments outside Denmark, Charlson´s comorbidity index must be filled in also.

Evaluation of late loco-regional RT associated morbidity

A secondary endpoint of this trial is arm lymphedema on the treated side after adjuvant radiation therapy. The definition of arm lymphedema is ≥10% increased arm circumference measured 15cm proximal and /or 10cm distal of the olecranon on the treated side compared to the contralateral side. If the patient uses an arm sleeve he/she is asked to not wear this sleeve 24 hours before measurement. This information is included in the patient information folder. There is no published data on how long time it takes for an arm edema to reach steady state after use of an sleeve, so 24 hours is an estimate. Also range of motion of the shoulders will be measured by investigating flexion / abduction of the upper arms. The late radiation induced morbidity will be evaluated and estimated in harmony with previous DBCG trials where the same endpoints have been reported, thus ensuring optimal conditions for comparison. Fibrosis estimates as tissue induration, telangiectasia, oedema of the breast/chest wall and dyspigmentation will be evaluated according to the LENT-SOMA scoring scale, and evaluation of the lumpectomy/mastectomy scar will be made according to a scale presented by Aaronson et al (22). Presence of ribs fractures are reported (must be documented on imaging). Based on Harris´ 4-point scale the global cosmetic result after breast conservation will be scored (23). A modification of the questionnaire designed, validated and used by Rune Gärtner et al will also be used in order to report on pain, swellings, discomfort and daily function (24). The patient

evaluates satisfaction on The Body Image Score (BIS) (25), whereto is added an extra question regarding clothing habits and furthermore also 2 more questions regarding satisfaction with the appearance of the treated breast after breast conservation with and without comparison to the opposite breast (26). In addition, we ask if the patient has had lipo-injection in her breast/breast region during follow up. This BIS is used in the DBCG HYPO, DBCG PBI, DBCG Skagen and DBCG Natural trials. In addition, questions regarding fear of cancer recurrence are asked.
Brachial plexopathy is a potential but seldom risk in patients treated with axillary lymph node dissection, taxan-based chemotherapy and loco-regional radiation therapy. It is however very difficult to distinguish between brachial plexopathy and the classical side effects from chemotherapy and surgical traumas (e.g. paresthesia). In this trial brachial plexopathy is therefore present if diagnosed by a neurologist. Thus if the patient has ipsilateral symptoms indicating plexopathy, the patient is recommended referred to a neurologist.

Reporting of morbidity is online via www.dbcg.dk, and should take place after every morbidity evaluation. Data is collected in DBCG which is a public register supervised by the Danish Regions. The study will comply with Danish legislation (Databeskyttelsesforordningen og Databeskyttelsesloven). The trial will be registered in the Region´s internal record of research projects. Data is managed according to the law about how to handle confidential information. Additional information regarding morbidity may be collected through questionnaires or the internet between two planned visits in the department. Staff involved in performing morbidity evaluation will be invited to participate in yearly workshops with focus on morbidity evaluation. The principal investigator is responsible for that. At these workshops patients with radiation related morbidity are invited for demonstration.

RT Quality Assurance (QA)

In a clinical RT trial, QA is of pivotal importance, and there will be an extensive reporting of the quality regarding target volume definition, treatment planning and dose distribution in targets and organs at risk. In addition, we will perform studies on robustness of proton versus photon therapy. This is carried out in all patients included in the trial. In order to start reporting results while still accruing patients, sub-studies are planned (see below). By participating in the trial, all patients accept extensive quality investigations in their treatment plan, and the sub-studies below are planned to allow for early reporting. The specific content of quality assurance will include new developments and ideas deemed to be relevant at the time of the quality assurance, since proton therapy for breast cancer at present is an experimental therapy and much development is ongoing.

Data from RT planning including imaging related to therapy of the breast cancer from all patients participating in the trial are part of the RT QA. There will be an extensive reporting of the quality regarding target volume definition and dose distribution in targets and organs at risk. In addition, we will perform several sub-studies when >100 patients have been treated:

1. Feasibility of proton versus photon RT, including reason for randomisation, accept of therapy, completing the RT as planned, geographical discrepancies having PT, delay in having RT
2. Robustness of PT and photon RT in patients operated with mastectomy, lumpectomy and reconstructions. All patients included in trial will have minimum weekly conebeam CT scans for image guided RT. The conebeam CT will be used to evaluate the influence from variations in anatomy during the RT course
3. Cine photos of intra fractional cardiac position measured on tangential photon fields. These evaluations aim at reporting incidental unplanned radiation of the heart. Results will be reported from patients treated at departments equipped with this technique. There is no extra radiation dose to the patient, and no prolonged treatment time.
4. All patients in this trial are treated with 40 Gy/15 fr or 50 Gy/25 fr or other DBCG accepted doses and fractionation schemes and protons and photons. There may be different biological effects from hypo- versus normofractionation, thus a higher

risk of reconstruction failure in patients treated with hypofractionation has been reported from an American breast cancer cohort treated with immediate breast reconstruction and post-operative radiation with either photon or proton (13). RT QA and influence from different fractionations will be investigated.

The details of these RT QA studies are planned by physicists involved in the trial (thus working at DCPT and/or referring RT departments), and the DBCG RT Committee must be informed and preferably involved in these studies. It is important to state that no extra imaging is carried out as part of this trial (except if the patient participates in the PET or the cardiac CT scan sub-studies). The imaging included in the RT QA analyses is imaging made related to treatment planning or verification.

Translational study, blood sample at study entry

PT is a new and relatively expensive treatment modality with a rapid increase in indications. There may be significant differences in the radiobiological mechanisms between protons and photons both for anti-cancer effects and for normal tissue toxicities, however, this is unknown. It is important to develop predictive factors for optimal selection of patients for PT, and a well-controlled clinical trial is a valuable resource to address these questions. All patients included in this trial are asked for minimum 1 baseline blood sample, however, some departments may collect more blood samples (the "large package", please, see page 26). Research will be carried out during accrual and follow up of the patients, and only for purposes within the scope of this trial. When all patients have been followed for 10 years, the trial closes and any remaining blood samples will be destroyed.

Translational study, economics

Information from all patients participating in the trial is used in this sub-study with no extra patient consent. Health economics of PT (phd project): PT is a financially demanding technology, and still with uncertainty about clinical gain relative to cost. It is therefore important to investigate whether the treatment is worthwhile, whether and for which patients it is cost-effective, and what is the expected impact on the health care budget (37). We plan to collect and analyse the relevant health economy parameters for all patients included in the randomised trial, including type, number and cost of equipment and personnel, patient population and treatment indications, time requirements, use of health related resources, disutility of care, return to work life etc, as described in details in (37). The data will form the basis of a cost accounting model, which together with the outcome data (morbidity, local control, quality of life, survival) will be used to estimate the cost-effectiveness of proton vs photons in early breast cancer. The study is planned and conducted in collaboration with leading health economists in DK and Europe through the EPTN (European Particle Therapy Network).

Translational study, radiosensitivity tumor and normal tissue

Whereas the potential benefit of PT is linked to a reduced dose and irradiated normal tissue volume, the risk of RT induced morbidity may also be associated with genetic based variation. Especially late morbidity such as fibrosis in breast cancer patients has been found to be associated with several genetic variations (39). A translational research program is therefore planned to analyse genetic variations in patients included in this trial in order to evaluate potential influence on the outcome. This will require genetic analysis of the blood samples from all included patients during the 10 year follow up (see section 7.0 below). The blood samples are stored in the Danish Cancer Biobank, except blood samples taken at Aarhus University Hospital are stored in a biobank at Experimental Clinical Oncology, Department of Oncology, Aarhus University Hospital.

Tumour response must also be adjusted for variation in genetic parameters, including stem cells markers and markers of intrinsic radiosensitivity. These can be derived from formalin fixed

paraffin embedded tumour material using techniques already established in our laboratory (40). The above genetic analyses will be carried out as a phd project at the dept. Experimental Clinical Oncology, AUH, and addressing normal tissue morbidity (in collaboration with the International Radiogenomic Collaboration) and tumour sensitivity (in collaboration with the DKTK – Deutsche Konsortium für translationale Krebsforschung), respectively.
By participating in the trial, the patient accepts that tissue from the primary tumour and later recurrences can be used for research purposes relevant to this trial e.g. investigation of predictive and prognostic markers of recurrence and gain from RT. However, this takes into account that there must always be enough tissue available in the Department of Pathology for further analyses related to future treatment of the patient.
The genetic investigations carried out in the DBCG Proton trial on blood samples and tissue samples will focus on a limited amount of genes involved in direct response to radiation therapy and indirect response to radiation therapy including inflammatory effects involved in radiation therapy. Mapping of all genes will not be done, since it is out of the scope of this trial.

7.0. SUB-STUDIES (extra patient consent is needed)

In the following, planned sub-studies are listed where a limited number of patients are invited to participate. Only patients providing specific consent for these sub-studies are investigated.

Evaluation of acute loco-regional RT associated morbidity (only patients who accept to participate)

Many women develop acute morbidity during a radiation therapy course. The acute morbidity is radiation dermatitis, itching, pain, fatigue, dyspnea, cough, pneumonitis, dysphagia, increased sensation of tightness of the shoulder and lymphedema. There are 2 internationally recognised systems for scoring acute radiation-induced morbidity, and they are the Common Toxicity Criteria (CTC), version 4.0 (34), and the Toxicity criteria of the RTOG and EORTC (35). Both systems overlap in scoring radiation dermatitis grades 0-4, whilst in the CTC system itching, pain, fatigue and dyspnoea, cough, pneumonitis, and dysphagia are also addressed.
Acute radiation-induced morbidity has not been systematically evaluated in Denmark since 1987, where evaluations were made in patients operated with mastectomy (36), except it was done in 40 patients per center in the DBCG Skagen trial 1 (data not reported 2019). In the report from 1987, 30% of women treated with normofractionated radiation therapy to a total of 42 Gy developed relatively severe erythema combined with dry desquamation (~grade 2), and 10% of the women developed moist desquamation (~grade 3). A study based on moderately hypofractionated breast radiation therapy reported radiation dermatitis grades 0, 1, 2, 3, and 4 in 10%, 69%, 18%, 4% and <1% of 339 women when they finished 42.5 Gy / 16 fractions (37).
In the DBCG PROTON Trial, 60 patients treated with protons and 60 patients treated with photons will be evaluated regarding acute morbidity.
All evaluations are made according to the scheme listed below, and breast photos are also taken at every evaluation. The scoring system is shown in Appendix 2.
For patients treated with 50 Gy / 25 fractions, the biologically effective dose per week is 10 Gy. For patients treated with 40 Gy / 15 fractions, based on an $\alpha/\beta = 10$, the dose per week is 14.14 Gy. It is a fact that acute radiation-induced morbidity is delayed in time about 2 weeks.
Irrespective of 15 or 25 fractions the evaluations listed in Table 6 are made weekly after start of radiation therapy. The evaluations are made in 120 patients total, 60 patients treated with protons and 60 patients treated with photons. After end of RT, evaluations including breast photos are made every 2 weeks as long as there are visible changes in the skin. When the visible skin changes have ceased, other acute morbidities (for example pruritus) may be evaluated by phone calls to the patient in 2 week intervals until the morbidity has reached the pre-radiation therapy level.
While the patient is at DCPT, the acute morbidities will be evaluated by the DCPT weekly, and each referring radiation centre will continue to evaluate the acute morbidity in the patients, when they have returned home.
All acute morbidities will be reported online to the DBCG database.
Pain will also be measured on a VAS score of 10 cm.
Patients invited to participate in this substudy should not be selected on patient or tumour characteristics, except perhaps geographical issues. Consecutively treated patients with

relatively short travel distance to the RT department may have a higher likelihood of completing the follow up visits as planned, so it is acceptable to select patients based on travel distance.

Table 6

	Before RT	**Weekly during RT (start at 2 weeks from first RT)**	**Every 2 weeks after end of RT until resolved**
Date	X	X	X
Radiation dermatitis	X	X	X
Pruritus	X	X	X
Pain	X	X	X
Fatigue	X	X	X
Dyspnea	X	X	X
Cough	X	X	X
Pneumonitis	X	X	X
Pneumonitis at 3 & 6 months after last RT (telephone call)			
Dysphagia	X	X	X
Arm lymphedema	X	X	X
Range of motion of shoulders	X	X	X
Photo	X	X	X

Frequency: For patients treated with 3 week RT, acute morbidity is evaluated at baseline, after 2 weeks, 3 weeks, and thereafter every 2 weeks. For patients treated with 5 week RT, acute morbidity is evaluated at baseline, at 2, 3, 4 and 5 weeks, and thereafter every 2 weeks.

Translational study, extra blood samples during follow up

To further investigate proton versus photon therapy and potential radiobiological differences regarding anti-cancer and late effects, serial blood samples are requested from patients who consent to participate in the ^{15}O-water-PET-CT study (N=50 patients) or in the Cardiac CT-lung study combined with Echocardiographies (N=150 patients). Only patients who participate in one of these two translational sub-studies are requested to give extra blood samples. Patients will be asked to give blood samples prospectively at end of RT, and year 1, 2, 3, 5 and 10 for translational research. Samples at these time points are taken from patients treated at AUH.

The hypothesis for these blood samples is that it may be possible to find markers of anti-cancer and late normal tissue effects and correlate the findings to changes detected in the imaging studies. Therefore, blood samples are always taken at the same time as for example heart CT and echocardiography to explore if early cardiac or lung disease may be detected in the blood.

Measurements on blood will include cardiac, pulmonary and inflammatory markers (e.g. Troponins, pro-BNP, Lymphocytes, cholesterols).

Funding has been obtained to cover costs of these blood samples in 200 patients treated at Aarhus University Hospital. Some of the other participating centers may also ask patients for research blood samples if they obtain funding for that, and the patient will be informed about this before randomisation and then invited to participate in this sub-study.

Translational study, heart CT scan and ECHO, RBE and DNA damage response

Cardiac and lung morbidities are endpoints in this trial. Serial heart CT scans and echo-cardiographies will be performed in a sub-set of 200 patients who consent for this sub-study. CT imaging will focus on both heart and lung, and the investigations are made 5 times during follow up: at baseline, at year 1, 3, 5, and 10. Each heart CT scan will be modified to include whole lung thus allowing for radiobiological research of lung morbidity also. One heart CT yields 0.8 mSv to the patient (see below). It is estimated that one heart CT scan in a 50 year old patient will induce a cancer in 1 out of 100,000 people. If one patient has all 5 planned heart CT scans, the total increased risk of cancer is 1 out of 10,000 people (Retningslinjer om anvendelse af ioniserende stråling I sundhedsvidenskabelige forsøg, NVK 2011). Funding has been obtained to cover the cost of these scans at AUH, so as of February 2020 only patients treated at AUH are invited for this sub-study. When CT scans from 50 of the planned 150 patients are available at 1 year follow up, investigations will be made to ensure that radiation changes in the scans are detectable and make sure that it is clinically meaningful to continue doing all these scans. All patients included in this sub-study will have echocardiography and electro-cardiography carried out before the heart CT scan. In addition, information on diabetes, dyslipidemia, hypertension, familiar disposition for cardiac disease (any AMI or by-pass surgery in first-degree (parents/siblings) family member <55 years if male and <65 years if female) and heart symptoms (angina at stress which disappears at rest and lasts <15 min) will be reported. All these informations are used when the cardiologist evaluates the heart CT scan and decides if there is a heart disease. In case there is a treatable heart disease, the cardiologist will inform the patient directly or inform the Dept of Oncology, AUH, and the patient will then be informed and guided to have treatment of the heart disease,
Eligible patients for the heart sub-study are patients with left-sided breast cancer, who do not have atrial fibrillation and are without asthma. The scans will be stored in a local database at the Dept Cardiology, AUH, and details from the evaluations will be reported to the West Danish Heart Registry and in that way be available to the patient if a heart disease is detected at any point of time later on. In this way, the heart imaging (echocardiography and heart CT) will not be destroyed at the end of this trial (at 10 years) because it may be of value to the patient if a heart disease occurs many years later.

The biological effects of proton therapy, in particular the potential impact of their increased relative biological effectiveness (RBE) are much less well understood than those of photons (50). The clinical treatment with protons rely on the RBE in order to convert from a physical dose to a biological equivalent dose. Currently, in proton therapy a constant RBE of 1.1 is generally used, as recommended by the International Commission on Radiation Units (51), meaning that a given proton dose is expected to be equivalent to a 10% higher X-ray dose for all tumors, tissues and doses. However, whether this is an adequate solution is under debate (52-54). The RBE is a complex figure, which is influenced by a number of factors. Tissue type, dose and fractionation, and LET (Linear Energy Transfer) are some of the factors known to affect the RBE (55, 56). The LET increases moderately through the Spread Out Bragg Peak (SOBP), with a substantial increase in the very distal edge of the SOBP. This has shown to translate into an increased distal edge RBE in vitro and in vivo in animal studies (57-61). This is a critical issue, as the distal edge of the SOBP may be situated in the surrounding normal tissue (62).
The final evaluation on the impact of variable proton RBE lies within the clinic and in clinical data, and clinical studies have indicated differential biological effect in different parts of the proton beam, as in the study by Peeler and colleges demonstrating a correlation between MRI changes and LET in pediatric patients treated for ependymoma (63). In this DBCG PROTON Trial cohort it is relevant to look into the effects in the normal tissue as a function of LET. For this purpose, the above mentioned heart CT scans are carried out at time points when radiation induced damage to lung and heart can be expected, and it is therefore planned to investigate all the cardiac CT scans also with this purpose. These can be correlated to the treatment plan, where the LET distribution can be assessed, as it was done on MRI scans in glioma patients after proton therapy in a recent study (64).
These heart CT scans will cause extra radiation to the patient at a magnitude of 0.8 mSv per scan. From the Danish Health authorities (Sundhedsstyrelsens Strålingsguiden Ioniserende Stråling 2013) the following is estimated: A Danish citizen on average receives 0.4 mSv yearly from food, 0.3 mSv from cosmic radiation, 0.3 mSv from the Earth and 2 mSv from Radon. A mammography causes 0.5 mSv. It is therefore considered acceptable for patients included in

this trial to have 6 heart CT scans during the 10 year follow up. If the heart CT or ECCO reveal cardiac disease which needs therapy, the patient will be informed and offered treatment.

Proton irradiation is inducing a differential biological response to photons in a number of parameters, and the DNA damage response has shown to induce different pathways, whether the DNA damage is induced by photons or protons (65). This opens for selective treatment of patients dependent on mutation status of the genes involved in DNA repair (66-67). The question is still open though, which genes are the best candidate biomarkers, and whether the differential radiobiology is reflected in the clinical situation, where more complex biological systems interplay. To answer this, parallel pre-clinical studies will be directed towards identifying genes as biomarkers for increased proton radiosensitivity in the normal tissue. To validate these in the DBCG PROTON Trial cohort, and to test for differential response following proton versus photon irradiation, part of the above mentioned blood samples will be used. It may be relevant to also investigate biopsies of both tumor and of normal skin (fibroblasts collected from the arm), but this will be applied for in another application for Ethical approval.

Translational study, ^{15}0-water-PET/CT and RT associated heart disease

^{15}O-water PET/CT for detection of RT-induced heart disease: RT is associated with cardiac complications when the heart is included in the RT field. Impairment of the coronary circulation occurs as direct vascular damage or accelerated atherosclerosis (41). Studies with conventional SPECT imaging have reported op to 60% new perfusions defects as early as 6 months after RT in patients receiving left breast RT (42, 43). These perfusion defects are typically limited to the anterior wall and apex (42), correlate with the volume of heart irradiated (44), remain relatively unchanged at 12- and 18-mo of follow-up compared with 6 mo after RT (43), and correlate with cardiovascular symptoms in those with new perfusion defects (45). However, none of the studies have used the golden standard ^{15}O-water PET/CT for absolute quantification of myocardial blood flow (MBF) and coronary flow reserve (CFR) for comparison of photon and proton RT induced heart disease.

Hypothesis: Proton radiation therapy will cause less myocardial perfusion abnormalities as compared to photon RT. MBF and CFR will be measured with ^{15}O-water PET/CT before, at 1 and 3 years after RT. Second, to prospectively compare RT-induced heart disease measured with ^{15}O-water PET/CT in patients with left-sided breast cancer treated with either PT or photon RT.

Methods: ^{15}O-labeled water PET/CT is the gold standard for absolute quantification of MBF (ml/min/g) and CFR (46-48). This quantification highly improves diagnostic accuracy as well as the prediction of major adverse cardiac events as compared to conventional SPECT imaging (49). The sensitivity, specificity, PPV, NPV and accuracy for ^{15}O-water PET/CT and SPECT are 87%, 84%, 81%, 89%, 85%, and 57%, 94%, 88%, 73%, and 77%, resp (49). We will use new high-end PET/CT scanners. Rest and adenosine stress ^{15}O-water PET/CT scans are done within 30 minutes. The rest and stress PET scans are performed after injection of 400 MBq of ^{15}O-water. Standard adenosine infusion is used for stress imaging. MBF, CFR, PTF and blood volume images are computer created. The radiation dose for cardiac ^{15}O-water water PET (rest and stress) is low (0,9 mSv). The Department of Nuclear medicine & PET Centre, AUH, has many years of experience with cardiac ^{15}O-water PET/CT.
Per 1 Gy mean heart dose (MHD), there is 10% increase in perfusion defects (43). MHD (PT)=0.5 Gy, MHD (photons)=4 Gy, thus 50 patients are needed to show less damage with PT. Only patients who consent to participate in this PET sub-study are eligible.

8.0 PUBLICATIONS

The results from this study will be published irrespective them being positive, negative or inconclusive. After approval of the study from the local scientific committee, the study will be registered on www.clinicaltrials.gov according to current recommendations.

Co-authorship will be given to the principal investigator and a representative from each participating department contributing with more than 5% of evaluable patients (2 representatives if contributing with more than 30%), and to the statistician who has contributed to collecting / validating and analysing data, and other persons who have contributed substantially to the implementation and/or evaluation of the trial. If some departments contribute with less than 5% of evaluable patients they can combine their contribution and share co-authorship alternating. The principal investigator is responsible for carrying out a draft manuscript for discussion among the co-authors. It is allowed to publish data regarding the primary and secondary endpoints from one´s own institution if the manuscript has been shown to the investigators of the other participating departments before submission, however, this must not take place before the results regarding the primary and secondary endpoints of the whole study cohort have been published. A number of planned analyses not infringing on the primary endpoint is, however, planned and publishable before the main results. Such studies must be described in detail in a separate statistical analysis plan and the associated trial statisticians will be involved to secure that they do not impact the primary endpoint and statistical design as described in this protocol. It is not accepted to publish results on ischaemic cardiac disease (the primary endpoint), nor second cancer or distant failures before these events have been reported at median 10 years follow up, because these events are the primary and major secondary endpoints. However, if independent data monitory committee or the trial group desires to publish early on these two events for reasons currently unknown, this can be done after mutual agreement and appropriate adjustment of the overall trial design. Thus, if for example results on acute radiation induced morbidity has been published from the whole cohort, it is allowed to report on acute morbidity from one´s own department, however, the results of acute morbidity from a single institution cannot be linked with other secondary endpoints of the randomised trial not yet published. Information, other than the primary and secondary endpoints, gathered from the study (for example through locally conducted studies regarding quality assurance of the radiotherapy or of the evaluation of morbidity) can be published from the institution(s) where this activity has been done, however, the principal investigator must be informed about this.
Co-authorship is given according to the Vancouver rules, however, these rules can be deviated from, for example should it happen that a person expected to be active turns out not to be active and/or an active person joins the study at a later time. This is to consider all involved parties. Projects defined at a later time and which uses some results / data from this trial can be published with the involved active persons only as co-authors together with the trial principal investigator only after accept from the protocol responsible investigators from the participating departments.
All publications from this trial should mention and thank relevant support including the support from DCCC Radiotherapy and DBCG (contact the trial principal investigator or DBCG for specific information), and The Novo Nordic Foundation.

9.0 ETHICAL CONSIDERATIONS

This trial is being conducted according to the 5th version of the Helsinki Declaration. The study can only start after the approval of the regional ethical committee for Region Midt.
The protocol contains experimental therapy using proton therapy. At each radiotherapy centre a protocol responsible person will take care that every patient is informed both verbally and in writing about the purpose and the course of the study. The patient will be informed about effects and side effects by participating in the study, and the patient will receive a written folder of information specifically regarding the study. This folder will meet the criteria for patient information in Denmark. The advantage for the patient by participating in the study is to receive more optimal dose coverage to target volumes and/or less dose to organs at risk. Based on already published data where proton therapy has been evaluated there is no expected increased radiation morbidity.
Both verbally and in writing the patient will be informed about the opportunity to withdraw consent at any time without giving a reason. If the patient chooses standard photon therapy this will consist of radiation therapy using same dose and fractionation as planned from the beginning. Before inclusion in the cohort study can take place the informed consent must be signed.

10.0 WITHDRAWAL OF CONSENT TO PARTICIPATE

Patients who for some reason do not receive the proton therapy should be treated according to best standard of care. Analysis of data will be according to the ”intention to treat” principle. Unless the patient does not want to, she must be followed up just like everybody else in the study with respect to the primary and secondary endpoints in the study. For patients who do not receive proton therapy, or who withdraw from the study after treatment, the date of withdrawal must be recorded in the DBCG database so that an updated status of participating patients can be made at every time desired.
To minimise withdrawal the patient should be carefully informed before inclusion in the study about the yearly detailed morbidity evaluation including photos. The patient may withdraw from the study at any time and she/he does not need to explain the reason

11.0 ECONOMICAL ISSUES

The initiative for this trial was taken by the principal investigator together with the DBCG RT Committee. The PI has written the majority of the protocol. The protocol has support from the DBCG Radiotherapy Committee and the study will be nationwide. All patients being candidates for this protocol are candidates for radiation therapy, thus the financial means are already available in the radiotherapy departments for the radiation therapy. The cost for morbidity evaluation will be paid by the different radiotherapy departments and is considered an operating cost.
The trial principal investigator has received funding from The Novo Nordic Foundation, 9.079.340 DKK. The grant reference number is NNF19OC0056870 in the call “Investigator Initiated Clinical Trials 2019”. A fee of 1400 DKK per randomised patient will be paid to the participating department, when the baseline blood sample has been stored. Each center decides how to spend the money, but it is possible to use the money for translational purposes, e.g. buy more blood samples and/or offer Cardiac CT and Echocardiograpies to the patients. Each center is responsible for the negotiations with other participating departments at their hospital.
Additional funding for sub-studies will be applied for. The trial has support from DCCC Radiotherapy. The principal investigator has no personal relations to Novo Nordisk. The protocol responsible doctor and physicist and the staff in each centre have no financial interests in the study.

12.0 REFERENCES

1: Ejlertsen B, Offersen BV, Overgaard J, Christiansen P, Jensen MB, Kroman N, Knoop AS, Mouridsen H. Forty years of landmark trials undertaken by the Danish Breast Cancer Cooperative Group (DBCG) nationwide or in international collaboration. Acta Oncol. 2018 Jan;57(1):3-12.

2: Thorsen LB, Offersen BV, Danø H, Berg M, Jensen I, Pedersen AN, Zimmermann SJ, Brodersen HJ, Overgaard M, Overgaard J. DBCG-IMN: A Population-Based Cohort Study on the Effect of Internal Mammary Node Irradiation in Early Node-Positive Breast Cancer. J Clin Oncol. 2016 Feb 1;34(4):314-20.

3: Poortmans PM, Collette S, Kirkove C, Van Limbergen E, Budach V, Struikmans H, Collette L, Fourquet A, Maingon P, Valli M, De Winter K, Marnitz S, Barillot I, Scandolaro L, Vonk E, Rodenhuis C, Marsiglia H, Weidner N, van Tienhoven G, Glanzmann C, Kuten A, Arriagada R, Bartelink H, Van den Bogaert W; EORTC Radiation Oncology and Breast Cancer Groups. Internal Mammary and Medial Supraclavicular Irradiation in Breast Cancer. N Engl J Med. 2015 Jul 23;373(4):317-27.

4: Whelan TJ, Olivotto IA, Parulekar WR, Ackerman I, Chua BH, Nabid A, Vallis KA, White JR, Rousseau P, Fortin A, Pierce LJ, Manchul L, Chafe S, Nolan MC, Craighead P, Bowen J, McCready DR, Pritchard KI, Gelmon K, Murray Y, Chapman JA,

Chen BE, Levine MN; MA.20 Study Investigators. Regional Nodal Irradiation in Early-Stage Breast Cancer. N Engl J Med. 2015 Jul 23;373(4):307-16.

5: Berg M, Lorenzen EL, Jensen I, Thomsen MS, Lutz CM, Refsgaard L, Nissen HD, Offersen BV. The potential benefits from respiratory gating for breast cancer patients regarding target coverage and dose to organs at risk when applying strict dose limits to the heart: results from the DBCG HYPO trial. Acta Oncol. 2018 Jan;57(1):113-119.

6: Darby SC, Ewertz M, McGale P, Bennet AM, Blom-Goldman U, Brønnum D, Correa C, Cutter D, Gagliardi G, Gigante B, Jensen MB, Nisbet A, Peto R, Rahimi K, Taylor C, Hall P. Risk of ischemic heart disease in women after radiotherapy for breast cancer. N Engl J Med. 2013 Mar 14;368(11):987-98.

7: Grantzau T, Thomsen MS, Væth M, Overgaard J. Risk of second primary lung cancer in women after radiotherapy for breast cancer. Radiother Oncol. 2014 Jun;111(3):366-73

8: Nilsson G, Witt Nyström P, Isacsson U, Garmo H, Duvernoy O, Sjögren I, Lagerqvist B, Holmberg L, Blomqvist C. Radiation dose distribution in coronary arteries in breast cancer radiotherapy. Acta Oncol. 2016 Aug;55(8):959-63.

9: Taylor C, Correa C, Duane FK, Aznar MC, Anderson SJ, Bergh J, Dodwell D, Ewertz M, Gray R, Jagsi R, Pierce L, Pritchard KI, Swain S, Wang Z, Wang Y, Whelan T, Peto R, McGale P; Early Breast Cancer Trialists' Collaborative Group. Estimating the Risks of Breast Cancer Radiotherapy: Evidence From Modern Radiation Doses to the Lungs and Heart and From Previous Randomized Trials. J Clin Oncol. 2017 May 20;35(15):1641-1649.

10: McGale P, Darby SC, Hall P, Adolfsson J, Bengtsson NO, Bennet AM, Fornander T, Gigante B, Jensen MB, Peto R, Rahimi K, Taylor CW, Ewertz M. Incidence of heart disease in 35,000 women treated with radiotherapy for breast cancer in Denmark and Sweden. Radiother Oncol. 2011 Aug;100(2):167-75.

11: Taylor CW, Brønnum D, Darby SC, Gagliardi G, Hall P, Jensen MB, McGale P, Nisbet A, Ewertz M. Cardiac dose estimates from Danish and Swedish breast cancer radiotherapy during 1977-2001. Radiother Oncol. 2011 Aug;100(2):176-83.

12: Stick LB, Yu J, Maraldo MV, Aznar MC, Pedersen AN, Bentzen SM, Vogelius IR. Joint Estimation of Cardiac Toxicity and Recurrence Risks After Comprehensive Nodal Photon Versus Proton Therapy for Breast Cancer. Int J Radiat Oncol Biol Phys. 2017 Mar 15;97(4):754-761.

13: Smith NL, Jethwa KR, Viehman JK, Harmsen WS, Gonuguntla K, Elswick SM, Grauberger JN, Amundson AC, Whitaker TJ, Remmes NB, Harless CA, Boughey JC, Nguyen MT, Park SS, Corbin KS, Mutter RW. Post-mastectomy intensity modulated proton therapy after immediate breast reconstruction: Initial report of reconstruction outcomes and predictors of complications. Radiother Oncol. 2019 Nov;140:76-83.

14: Luo L, Cuaron J, Braunstein L, Gillespie E, Kahn A, McCormick B, Mah D, Chon B, Tsai H, Powell S, Cahlon O. Early outcomes of breast cancer patients treated with post-mastectomy uniform scanning proton therapy. Radiother Oncol. 2019 Mar;132:250-256.

15: Verma V, Iftekaruddin Z, Badar N, Hartsell W, Han-Chih Chang J, Gondi V, Pankuch M, Gao M, Schmidt S, Kaplan D, McGee L. Proton beam radiotherapy as part of comprehensive regional nodal irradiation for locally advanced breast cancer. Radiother Oncol. 2017 May;123(2):294-298.

16: Bradley JA, Dagan R, Ho MW, Rutenberg M, Morris CG, Li Z, Mendenhall NP. Initial Report of a Prospective Dosimetric and Clinical Feasibility Trial Demonstrates the Potential of Protons to Increase the Therapeutic Ratio in Breast Cancer Compared With Photons. Int J Radiat Oncol Biol Phys. 2016 May 1;95(1):411-21.

17: MacDonald SM, Jimenez R, Paetzold P, Adams J, Beatty J, DeLaney TF, Kooy H, Taghian AG, Lu HM. Proton radiotherapy for chest wall and regional lymphatic radiation; dose comparisons and treatment delivery. Radiat Oncol. 2013 Mar 24;8:71.

18: Bekelman JE, Lu H, Pugh S, Baker K, Berg CD, de Gonzalez AB, Braunstein LZ, Bosch W, Chauhan C, Ellenberg S, Fang LC, Freedman GM, Hahn EA, Haffty BG, Khan AJ, Jimenez RB, Kesslering C, Ky B, Lee C, Lu HM, Mishra MV, Mullins CD, Mutter RW, Nagda S, Pankuch M, Powell SN, Prior FW, Schupak K, Taghian AG, Wilkinson JB, MacDonald SM, Cahlon O; RadComp (Radiotherapy Comparative Effectiveness Consortium). Pragmatic randomised clinical trial of proton versus photon therapy for patients with non-metastatic breast cancer: the Radiotherapy Comparative Effectiveness (RadComp) Consortium trial protocol. BMJ Open. 2019 Oct 15;9(10):e025556.

19: Stovall M, Smith SA, Langholz BM, Boice JD Jr, Shore RE, Andersson M, Buchholz TA, Capanu M, Bernstein L, Lynch CF, Malone KE, Anton-Culver H, Haile RW, Rosenstein BS, Reiner AS, Thomas DC, Bernstein JL; Women's Environmental, Cancer, and Radiation Epidemiology Study Collaborative Group. Dose to the contralateral breast from radiotherapy and risk of second primary breast cancer in the WECARE study. Int J Radiat Oncol Biol Phys. 2008 Nov 15;72(4):1021-30.

20: Rehammar JC, Jensen MB, McGale P, Lorenzen EL, Taylor C, Darby SC, Videbæk L, Wang Z, Ewertz M. Risk of heart disease in relation to radiotherapy and chemotherapy with anthracyclines among 19,464 breast cancer patients in Denmark, 1977-2005. Radiother Oncol. 2017 May;123(2):299-305.

21: Underwood TSA, Grassberger C, Bass R, MacDonald SM, Meyersohn NM, Yeap BY, Jimenez RB, Paganetti H. Asymptomatic Late-phase Radiographic Changes Among Chest-Wall Patients Are Associated With a Proton RBE Exceeding 1.1. Int J Radiat Oncol Biol Phys. 2018 Jul 15;101(4):809-819.

22: Aaronson NK, Bartelink H, van Dongen JA, van Dam FS. Evaluation of breast conserving therapy: clinical, methodological and psychosocial perspectives. Eur J Surg Oncol. 1988 Apr;14(2):133-40.

23: Harris JR, Levene MB, Svensson G, Hellman S. Analysis of cosmetic results following primary radiation therapy for stages I and II carcinoma of the breast. Int J Radiat Oncol Biol Phys. 1979 Feb;5(2):257-61.

24: Gärtner R, Jensen MB, Kronborg L, Ewertz M, Kehlet H, Kroman N. Self-reported arm-lymphedema and functional impairment after breast cancer treatment--a nationwide study of prevalence and associated factors. Breast. 2010 Dec;19(6):506-15.

25: Hopwood P, Fletcher I, Lee A, Al Ghazal S. A body image scale for use with cancer patients. Eur J Cancer. 2001 Jan;37(2):189-97.

26: Lyngholm CD, Christiansen PM, Damsgaard TE, Overgaard J. Long-term follow-up of late morbidity, cosmetic outcome and body image after breast conserving therapy. A study from the Danish Breast Cancer Cooperative Group (DBCG). Acta Oncol. 2013 Feb;52(2):259-69.

27: Offersen BV, Boersma LJ, Kirkove C, Hol S, Aznar MC, Sola AB, Kirova YM,

Pignol JP, Remouchamps V, Verhoeven K, Weltens C, Arenas M, Gabrys D, Kopek N, Krause M, Lundstedt D, Marinko T, Montero A, Yarnold J, Poortmans P. ESTRO consensus guideline on target volume delineation for elective radiation therapy of early stage breast cancer, version 1.1. Radiother Oncol. 2016 Jan;118(1):205-8.

28: Offersen BV, Boersma LJ, Kirkove C, Hol S, Aznar MC, Biete Sola A, Kirova YM, Pignol JP, Remouchamps V, Verhoeven K, Weltens C, Arenas M, Gabrys D, Kopek N, Krause M, Lundstedt D, Marinko T, Montero A, Yarnold J, Poortmans P. ESTRO consensus guideline on target volume delineation for elective radiation therapy of early stage breast cancer. Radiother Oncol. 2015 Jan;114(1):3-10.

29: Kaidar-Person O, Vrou Offersen B, Hol S, Arenas M, Aristei C, Bourgier C, Cardoso MJ, Chua B, Coles CE, Engberg Damsgaard T, Gabrys D, Jagsi R, Jimenez R, Kirby AM, Kirkove C, Kirova Y, Kouloulias V, Marinko T, Meattini I, Mjaaland I, Nader Marta G, Witt Nystrom P, Senkus E, Skyttä T, Tvedskov TF, Verhoeven K, Poortmans P. ESTRO ACROP consensus guideline for target volume delineation in the setting of postmastectomy radiation therapy after implant-based immediate reconstruction for early stage breast cancer. Radiother Oncol. 2019 Aug;137:159-166.

30: Feng M, Moran JM, Koelling T, Chughtai A, Chan JL, Freedman L, Hayman JA, Jagsi R, Jolly S, Larouere J, Soriano J, Marsh R, Pierce LJ. Development and validation of a heart atlas to study cardiac exposure to radiation following treatment for breast cancer. Int J Radiat Oncol Biol Phys. 2011 Jan 1;79(1):10-8.

31: Lorenzen EL, Taylor CW, Maraldo M, Nielsen MH, Offersen BV, Andersen MR, O'Dwyer D, Larsen L, Duxbury S, Jhitta B, Darby SC, Ewertz M, Brink C. Inter-observer variation in delineation of the heart and left anterior descending coronary artery in radiotherapy for breast cancer: a multi-centre study from Denmark and the UK. Radiother Oncol. 2013 Aug;108(2):254-8.

32: Gregoire V et al, ICRU 38. Prescribing, recording and reporting photon-beam intensity-modulated radiotherapy (IMRT). Journal of ICRU 10, 2010.

33: Delanian S, Lefaix JL, Pradat PF. Radiation-induced neuropathy in cancer survivors. Radiother Oncol. 2012 Dec;105(3):273-82.

34: Common terminology criteria for adverse events (CTCAE) version 4.0, 2009

35: Cox JD, Stetz J, Pajak TF. Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC). Int J Radiat Oncol Biol Phys. 1995 Mar 30;31(5):1341-6.

36: Overgaard M, Bentzen SM, Christensen JJ, Madsen EH. The value of the NSD formula in equation of acute and late radiation complications in normal tissue following 2 and 5 fractions per week in breast cancer patients treated with postmastectomy irradiation. Radiother Oncol. 1987 May;9(1):1-11.

37: Plataniotis GA, Theofanopoulou MA, Sotiriadou K, Kyrgias G. Hypofractionated radiotherapy for breast cancer patients treated by breast-conserving surgery: short-term morbidity and preliminary results. Breast Cancer. 2010;17(1):42-7.

38: Lievens Y, Nagels K. Economic data for particle therapy: Dealing with different needs in a heterogeneous landscape. Radiother Oncol. 2018 Jul;128(1):19-25.

39: Alsner J, Andreassen CN, Overgaard J. Genetic markers for prediction of normal tissue toxicity after radiotherapy. Semin Radiat Oncol. 2008

Apr;18(2):126-35.

40: Tramm T, Mohammed H, Myhre S, Kyndi M, Alsner J, Børresen-Dale AL, Sørlie T, Frigessi A, Overgaard J. Development and validation of a gene profile predicting benefit of postmastectomy radiotherapy in patients with high-risk breast cancer: a study of gene expression in the DBCG82bc cohort. Clin Cancer Res. 2014 Oct 15;20(20):5272-80.

41: Dreyfuss AD, Bravo PE, Koumenis C, Ky B. Precision Cardio-Oncology. J Nucl Med. 2019 Apr;60(4):443-450.

42: Hardenbergh PH, Munley MT, Bentel GC, Kedem R, Borges-Neto S, Hollis D, Prosnitz LR, Marks LB. Cardiac perfusion changes in patients treated for breast cancer with radiation therapy and doxorubicin: preliminary results. Int J Radiat Oncol Biol Phys. 2001 Mar 15;49(4):1023-8.

43: Lind PA, Pagnanelli R, Marks LB, Borges-Neto S, Hu C, Zhou SM, Light K, Hardenbergh PH. Myocardial perfusion changes in patients irradiated for left-sided breast cancer and correlation with coronary artery distribution. Int J Radiat Oncol Biol Phys. 2003 Mar 15;55(4):914-20.

44: Marks LB, Yu X, Prosnitz RG, Zhou SM, Hardenbergh PH, Blazing M, Hollis D, Lind P, Tisch A, Wong TZ, Borges-Neto S. The incidence and functional consequences of RT-associated cardiac perfusion defects. Int J Radiat Oncol Biol Phys. 2005 Sep 1;63(1):214-23.

45: Xu X et al, Symptomatic cardiac events following radiation. Clin Breast Cancer 2003; 4: 193-197

46: Bergmann SR, Fox KA, Rand AL, McElvany KD, Welch MJ, Markham J, Sobel BE. Quantification of regional myocardial blood flow in vivo with H215O. Circulation. 1984 Oct;70(4):724-33.

47: Herrero P, Markham J, Bergmann SR. Quantitation of myocardial blood flow with H2 15O and positron emission tomography: assessment and error analysis of a mathematical approach. J Comput Assist Tomogr. 1989 Sep-Oct;13(5):862-73.

48: Danad I, Raijmakers PG, Driessen RS, Leipsic J, Raju R, Naoum C, Knuuti J, Mäki M, Underwood RS, Min JK, Elmore K, Stuijfzand WJ, van Royen N, Tulevski II, Somsen AG, Huisman MC, van Lingen AA, Heymans MW, van de Ven PM, van Kuijk C, Lammertsma AA, van Rossum AC, Knaapen P. Comparison of Coronary CT Angiography, SPECT, PET, and Hybrid Imaging for Diagnosis of Ischemic Heart Disease Determined by Fractional Flow Reserve. JAMA Cardiol. 2017 Oct 1;2(10):1100-1107.

49: Driessen RS, Raijmakers PG, Stuijfzand WJ, Knaapen P. Myocardial perfusion imaging with PET. Int J Cardiovasc Imaging. 2017 Jul;33(7):1021-1031.

50: Lühr A, von Neubeck C, Pawelke J, Seidlitz A, Peitzsch C, Bentzen SM, Bortfeld T, Debus J, Deutsch E, Langendijk JA, Loeffler JS, Mohan R, Scholz M, Sørensen BS, Weber DC, Baumann M, Krause M; “Radiobiology of Proton Therapy”. "Radiobiology of Proton Therapy": Results of an international expert workshop. Radiother Oncol. 2018 Jul;128(1):56-67.

51: ICRU Report 78. Prescribing, recording and reporting proton-beam therapy. J ICRU 2007

52: Ödén J, DeLuca PM Jr, Orton CG. The use of a constant RBE=1.1 for proton radiotherapy is no longer appropriate. Med Phys. 2018 Feb;45(2):502-505.

53: Jones B. Why RBE must be a variable and not a constant in proton therapy. Br J Radiol. 2016 Jul;89(1063):20160116.

54: Buchsbaum JC, McDonald MW, Johnstone PA, Hoene T, Mendonca M, Cheng CW, Das IJ, McMullen KP, Wolanski MR. Range modulation in proton therapy planning: a simple method for mitigating effects of increased relative biological effectiveness at the end-of-range of clinical proton beams. Radiat Oncol. 2014 Jan 2;9:2.

55: Paganetti H, van Luijk P. Biological considerations when comparing proton therapy with photon therapy. Semin Radiat Oncol. 2013 Apr;23(2):77-87.

56: Tommasino F, Durante M. Proton radiobiology. Cancers (Basel). 2015 Feb 12;7(1):353-81

57: Bettega D, Calzolari P, Chauvel P, Courdi A, Herault J, Iborra N, Marchesini R, Massariello P, Poli GL, Tallone L. Radiobiological studies on the 65 MeV therapeutic proton beam at Nice using human tumour cells. Int J Radiat Biol. 2000 Oct;76(10):1297-303.

58: Sørensen BS, Overgaard J, Bassler N. In vitro RBE-LET dependence for multiple particle types. Acta Oncol. 2011 Aug;50(6):757-62.

59: Britten RA, Nazaryan V, Davis LK, Klein SB, Nichiporov D, Mendonca MS, Wolanski M, Nie X, George J, Keppel C. Variations in the RBE for cell killing along the depth-dose profile of a modulated proton therapy beam. Radiat Res. 2013 Jan;179(1):21-8.

60: Wouters BG, Skarsgard LD, Gerweck LE, Carabe-Fernandez A, Wong M, Durand RE, Nielson D, Bussiere MR, Wagner M, Biggs P, Paganetti H, Suit HD. Radiobiological intercomparison of the 160 MeV and 230 MeV proton therapy beams at the Harvard Cyclotron Laboratory and at Massachusetts General Hospital. Radiat Res. 2015 Feb;183(2):174-87. doi: 10.1667/RR13795.1. Epub 2015 Jan 14. Erratum in: Radiat Res. 2015 Apr;183(4):e51. PubMed PMID: 25587741.

61: Sørensen BS. Commentary: RBE in proton therapy - where is the experimental in vivo data? Acta Oncol. 2019 Oct;58(10):1337-1339.

62: Paganetti H, Blakely E, Carabe-Fernandez A, Carlson DJ, Das IJ, Dong L, Grosshans D, Held KD, Mohan R, Moiseenko V, Niemierko A, Stewart RD, Willers H. Report of the AAPM TG-256 on the relative biological effectiveness of proton beams in radiation therapy. Med Phys. 2019 Mar;46(3):e53-e78.

63: Peeler CR, Mirkovic D, Titt U, Blanchard P, Gunther JR, Mahajan A, Mohan R, Grosshans DR. Clinical evidence of variable proton biological effectiveness in pediatric patients treated for ependymoma. Radiother Oncol. 2016 Dec;121(3):395-401.

64: Eulitz J, Troost EGC, Raschke F, Schulz E, Lutz B, Dutz A, Löck S, Wohlfahrt P, Enghardt W, Karpowitz C, Krause M, Lühr A. Predicting late magnetic resonance image changes in glioma patients after proton therapy. Acta Oncol. 2019 Oct;58(10):1536-1539.

65: Grosse N, Fontana AO, Hug EB, Lomax A, Coray A, Augsburger M, Paganetti H, Sartori AA, Pruschy M. Deficiency in homologous recombination renders Mammalian cells more sensitive to proton versus photon irradiation. Int J Radiat Oncol Biol Phys. 2014 Jan 1;88(1):175-81.

66: Fontana AO, Augsburger MA, Grosse N, Guckenberger M, Lomax AJ, Sartori AA, Pruschy MN. Differential DNA repair pathway choice in cancer cells after proton- and photon-irradiation. Radiother Oncol. 2015 Sep;116(3):374-80.

67: Willers H, Gheorghiu L, Liu Q, Efstathiou JA, Wirth LJ, Krause M, von Neubeck C. DNA Damage Response Assessments in Human Tumor Samples Provide Functional Biomarkers of Radiosensitivity. Semin Radiat Oncol. 2015 Oct;25(4):237-50.